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VOLUME 43 NOVEMBER 1953 NUMBER 11

Phytopathology

An International Journal

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

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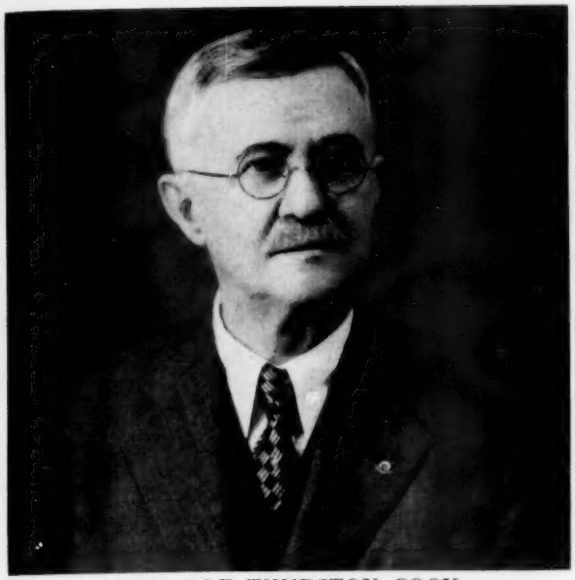
MELVILLE THURSTON COOK, 1869-1952

C. W. Edgerton



MELVILLE THURSTON COOK
1869-1952

Melville Thurston Cook, a life member of the American Phytopathological Society, died in Washington, D. C., August 11, 1952 at the age of 82. His active service in teaching and research covered a period of more than 50 years. He was one of the few remaining of that group that in the early years of the present century firmly established plant pathology as an important member of the plant sciences in the United States.

Dr. Cook was born on September 20, 1869, in Coffeen, Illinois, where his father practiced medicine. After finishing his elementary education, he attended DePauw University for 3 years, then went to Leland Stanford University where he received the bachelor of arts degree in 1894. A year's teaching in the Vandalia High School was followed by graduate work at DePauw University. He received his master of arts degree from DePauw in 1902 and in 1904 his doctor of philosophy degree from Ohio State University. In 1940, the honorary degree of doctor of science was conferred upon him by the University of Puerto Rico and by DePauw University.

Dr. Cook held many positions in the fields of biology, botany, and plant pathology including: instructor in biology, DePauw, 1895; professor of biology, DePauw, 1897-1904; plant pathologist, Central Agronomy Station, Santiago de las Vegas, Cuba, 1904-1906; plant pathologist, Delaware Agricultural Experiment Station, 1907-1911; professor of botany, Rutgers College, and plant pathologist of the New Jersey Agricultural Experiment Station, 1911-1923; plant pathologist and at times acting director of the Insular Experiment Station, Rio Piedras, Puerto Rico, 1923-1940. He retired in 1940 because of ill health, but in 1942 came to Louisiana State University as a temporary, part-time visiting professor with most of his time available for research. He remained in Louisiana until June, 1952, when he resigned, knowing that his health would not permit him to continue.

In 1894, Dr. Cook married Dora Reavill, who survives him and is at present living in Hyattsville, Maryland, with their son. Surviving also are their 3 children: Mrs. Elizabeth Ross, of Larchmont, New York; Harvey R. Cook, of Detroit, Michigan; and Dr. Harold T. Cook, plant pathologist in the United States Department of Agriculture at Beltsville, Maryland.

Dr. Cook was a charter member of the American Phytopathological Society, attended practically all the annual meetings, and took a prominent part in its activities. He was vice president in 1916 and president in 1917. He was a Fellow of the American Association for the Advancement of Science and a member of the Botanical Society of America, the American Mycological Society, and Sigma Xi.

His researches covered a wide field. At first as a graduate student, he was primarily interested in the cause and development of plant galls. Later, as his positions demanded, he became mainly interested in plant diseases. In Delaware and New Jersey, truck crops and fruits received much of his attention. When he went to Puerto Rico, his interest shifted to tropical crops such as sugarcane, citrus fruits, and coffee. The information he acquired during his 17 years in Puerto Rico made him an authority on diseases of tropical plants.

For many years he was greatly interested in virus diseases. While at Louisiana State University he published *Viruses and Virus Diseases of Plants*, which has been extremely useful to plant pathologists.

Later, he became interested in Synchytriums, a group of fungi not very well known at the time. He studied not only the species which he found in Louisiana but many others from various parts of the world. He described a number of new species and was responsible for an awakened interest in this group.

Other books published by Dr. Cook included: *The Diseases of Tropical Plants*, 1913; *Applied Economic Botany*, 1919; *College Botany*, 1920; *Enfermedades de las Plantas Económicas de las Antillas*, 1939. He also published more than 400 articles in scientific journals and as Experiment Station bulletins. For a number of years while in Puerto Rico, he edited the Journal of Agriculture of the University of Puerto Rico, a journal of great value to pathologists in tropical and subtropical countries.

Dr. Cook was a kindly man. He made numerous friends wherever he went. He wanted to serve and be useful. His office door was always open and he was never too busy or too ill to assist or to advise anyone who wanted advice. Thus will he be remembered.

JAMES STEWART WIANT, 1900-1952

Harold T. Cook

James Stewart Wiant, senior pathologist in charge of the U. S. Department of Agriculture Market Pathology Laboratory in New York City, died on March 15, 1952, at his home in Westfield, New Jersey, after an illness of 9 months.

Dr. Wiant was born at Dorranceton, Pennsylvania October 5, 1900. He was the son of David Wiant, a school teacher and farmer, and May Koons Wiant. In 1907 the family moved to Huntington Mills, Pennsylvania, and about 3 years later to a nearby farm. From his father and his rural environment he acquired an early interest in nature study, which he retained the rest of his life.

From 1913 to 1917 he took a teacher training course at Bloomsburg State Normal School and for the next 3 years he was on the faculty as "assistant in biological sciences." During that period he took additional courses to prepare for college and studied 2 summers at the Marine Biological Laboratories at Cold Spring Harbor, New York. He entered Pennsylvania State College in September 1920 and graduated in 1924. From 1924 to 1928 he was a graduate student in plant pathology at Cornell University from which he obtained the Ph.D. degree in 1928.

Dr. Wiant was assistant agronomist and plant pathologist at the Wyoming Agricultural Experiment Station from 1928 to 1931. While there he developed a scientific seed potato certification program for Wyoming, which included growing samples of each farmer's seed in the South during the winter. He also taught a course in plant pathology and did research on bacterial wilt of alfalfa.

In 1931 he was appointed associate plant pathologist in the U. S. Department of Agriculture and assigned to the Market Pathology Laboratory in New York City. Advancing through the grades to senior pathologist, he was head of the New York Laboratory from 1947 until his death.

Dr. Wiant made many important contributions in the field of transportation, storage, and market diseases of fruits and vegetables and became a recognized leader in this field.

Although confined to his room for 9 months before his death, Dr. Wiant directed the work of his laboratory by telephone, letter, and personal conferences with members of his staff. During that period he also analyzed considerable data and prepared several manuscripts.

Dr. Wiant took an active part in church, school, and civic groups. His hobby was nature study, especially ornithology and he was advisor on mushrooms to the Trailside Museum of the Union County (New Jersey) Park System. He was a member of Alpha Zeta, Phi Kappa Phi, Gamma Sigma Delta, Sigma Xi, the American Phytopathological Society and a fellow of the American Association for the Advancement of Science.

JAMES STEWART WIANT
1900-1952

In June 1930, he married Mary Powell, who graduated from Bloomsburg State Normal School in his class. Besides his widow, a daughter, Betty Joan, and a son, James Robinson, survive him.

The following are a few of Dr. Wiant's more important publications:

1929 The Rhizoctonia damping-off of conifers, and its control by chemical treatment of the soil. N.Y. (Cornell) Agr. Exp. Sta. Mem. 124: 64 pp.

1930 Certain aspects of potato certification in Wyoming. Amer. Potato Jour. 7: 30-33.

1937 Investigations of the market diseases of cantaloups and honey dew and honey ball melons. U. S. Dept. Agr., Tech. Bul. 573. 47 pp., illus.

1938 Market diseases of fruits and vegetables: crucifers and cucurbits. U. S. Dept. Agr., Misc. Pub. 292, 74 pp., illus. (With G. B. Ramsey and G. K. K. Link).

1938 Market-storage studies of honey dew melons and cantaloups. U. S. Dept. Agr., Tech. Bul. 613, 18 pp., illus.

1939 White rust of spinach. Phytopathology 29: 616-623, illus. (With S. S. Ivanoff and J. A. Stevenson).

1941 Market disease of fruit and vegetables: asparagus, onions, beans, peas, carrots, celery, and related vegetables. U. S. Dept. Agr. Misc. Publ. 440, 70 pp. (With G. B. Ramsey)

1944 Market diseases of fruits and vegetables: beets, endive, escarole, globe artichokes, lettuce, rhubarb, spinach, Swiss chard, and sweet-potatoes. U. S. Dept. Agr. Misc. Pub. 541, 40 pp. (With G. B. Ramsey).

1948 Spoilage of fresh fruits and vegetables in rail shipments unloaded at New York City, 1935-42. U. S. Dept. Agr. Cir. 773, 62 pp. (With C. O. Bratley).

1952 Market diseases of tomatoes, peppers and eggplants. U. S. Dept. Agr. Agricultural Handbook 28: 54 pp. (With G. B. Ramsey and L. P. McColloch).

SOIL FUMIGATION AGAINST VERTICILLIUM ALBO-ATRUM[1]

Stephen Wilhelm and John Ferguson

The vascular pathogen *Verticillium albo-atrum* Rke. et. Bert. has an extensive host range which includes important crops, and is widespread in California soils. Its microsclerotia, which form in senescent and dead plant tissue after pathogenesis, are notably tolerant of adverse environmental conditions, especially of drying and high temperatures, and undoubtedly enable this fungus to persist for long periods in soils (8 years in one instance studied). Though apparently unable to make significant active saprophytic growth in soil (9), yet *Verticillium* withstands increased biological activity attendant upon additions of considerable quantities of certain amendments (8).

This paper, an expansion of an earlier brief report (2) deals with the lethal action in soil of certain volatile liquid fumigants on *Verticillium*. It presents post laboratory methods of evaluating in soil the fungicidal potentialities of chemicals selected as promising by screening tests in the laboratory. The methods are believed to be applicable to the general problem of studying soil fumigants.

Many chemicals show definite promise as soil fungicides in laboratory tests, but fail when tested in the field. Failure in the field may result from any of the many interacting factors which attend exposure of the chemical to the soil, such as absorption or other types of inactivation by the soil, too slow or too rapid diffusion, both of which may be affected by soil type, organic matter content, temperature, and moisture. These factors may greatly restrict the usefulness of laboratory information.

USE OF MICROSCLEROTIA IN A NATURAL MEDIUM TO MEASURE SURVIVAL OF VERTICILLIUM IN TREATED SOILS —The chemicals were injected into composted soil in 32 gal. cans in which test inoculum of *Verticillium* was placed at measured depths from 1½ to 24 in. Reasonably constant soil compaction was achieved by using equal amounts of loose soil from a large pile for each horizon. The test inoculum was prepared by dipping dried, sterilized tomato stem pieces into a heavy spore suspension obtained from single-spore cultures of a strain of *V. albo-atrum* pathogenic to tomato. The tomato stem pieces thus seeded were placed on water agar until abundant sclerotia developed in and on them. Because preliminary tests revealed that drying of the tomato pieces containing sclerotia for as long as 4 months caused no appreciable change in susceptibility to chloropicrin, the test pieces were used well within that time limit, usually about 3 weeks after seeding. For a particular experiment, the test pieces were tied to a stiff paper label on which was indicated the chemical used, depth of inoculum, exposure time, and dosage applied.

The inoculum thus tagged was placed at different known levels in the soil. The cans with a surface area of 2 sq. ft. (20 in. in diameter, 24 in. in depth) were filled to contain either 2 or 4 cu. ft. of soil. Four pieces of test inoculum spaced 90° apart and 9 in. from the center were placed at each depth. The fumigant was injected into the soil in the center of the can at various depths. Deep injections were made through glass tubes, shallow injections with a hypodermic needle. The holes made for injecting the chemicals were filled immediately. The soil was not sealed as with water nor covered directly, but a loose fitting metal lid was placed over the top of the can. A non-fumigated and a chloropicrin fumigated control was run for each series of tests. After the exposure period the soil was taken from the cans and put through a ¼ in. mesh screen to remove the test pieces. Each test piece thus retrieved was placed in a sterile Petri dish, washed with sterile water, and plated out on 2 per cent water agar to which barley straw previously sterilized with propylene oxide had been added. This barley straw affords a natural substrate insofar as it has not been heat sterilized (5). Though typical conidial development usually occurred within 3 days from any living sclerotia, final results were based upon the production by *Verticillium* of its

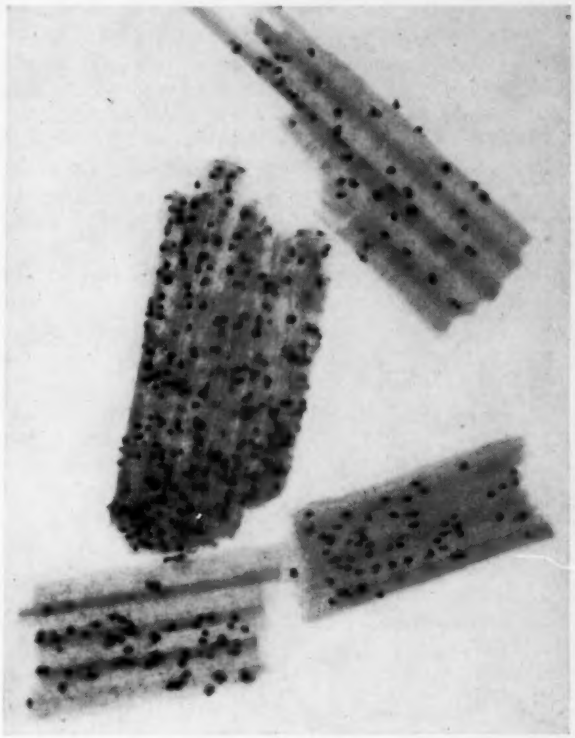


FIG. 1. Microsclerotia of *Verticillium albo-atrum* as they develop in the unheated propylene-oxide-fumigated pieces of barley straw on water agar. Their distinctiveness affords a ready means of detecting the fungus.

[1] Accepted for publication July 6, 1953.

characteristic microsclerotia in the straw fragments in the agar. Since many different fungi were at one time or another found growing from the test pieces, the distinctive characteristics of the Verticillium microsclerotia which developed in the straw provided an easy and certain method of detection (Fig. 1).

The other organisms found associated with the test pieces varied. A distinctly different flora occurred on test pieces containing killed, as against surviving, sclerotia. For instance on killed Verticillium inoculum, species of *Chaetomium, Trichoderma, Gliocladium, Graphium,* actinomycetes, and bacteria, especially pink and yellow colonies, predominated. Occasionally species of *Mucor, Thamnidium, Fusarium, Melanospora, Sordaria, Arthrobotrys, Pyronema, Trichothecium, Epicoccum, Stachybotrys,* and *Dictyostelium* were encountered. Test inoculum not killed yielded typically an abundance of bacteria, free-living nematodes, *Dictyostelium* and other slime molds, and few fungi, in fact rarely species of *Chaetomium, Gliocladium* or *Trichoderma.* One or 2 drops of pure ethylene dibromide placed on the cover plate of a Petri dish culture would usually rid the culture of the objectionable nematodes and permit normal development of *Verticillium* and other fungi. The predominant flora was also governed somewhat by the chemical used. For instance *Chaetomium* species were predominant after chloropicrin treatments, whereas *Trichoderma* appeared predominant after allyl bromide.

Soil and air temperatures were recorded automatically during the fumigation period. The moisture content was determined on a dry weight basis by dividing the loss in weight upon drying by the dry weight of the soil and was found to be fairly constant throughout the tests. The more promising of the chemicals were tested outside during winter and summer in order to take advantage of both low and moderate soil temperatures.

The following chemicals, because of reported fungicidal action, or of close chemical relationship to known fungicides were tested in at least 3 concentrations: allyl acetate, allyl alcohol, allyl bromide, allyl chloride, allyl formate, ammonium hydrosulfide, carbon disulfide, chlorobromopropene (commercial CBP-55), chloropicrin (commercial Larvacide), ethylene dibromide, formaldehyde, isocaproic acid, methylol, nitroethane, nitromethane, o-chlorophenol, pentachloroethane, propylene bromide, propylene oxide, tetrachloroethane, and tetrachloroethylene.

CHLOROPICRIN, CHLOROBROMOPROPENE AND ALLYL BROMIDE WERE EFFECTIVE AGAINST VERTICILLIUM.—Of the chemicals tested, commercial Larvacide (chloropicrin), CBP-55 (approximately 55 per cent 1 chloro-3-bromopropene-1, (4) and allyl bromide were effective against *Verticillium.* Minimal dosage required to kill *Verticillium* to a depth of 12 in. was expressed as cc. of chemical per sq. ft. of soil area.

Data typical of the results obtained from tests with chloropicrin in which time, injection depth, and dosage were studied appear in Table 1. At 1 cc. per sq. ft. only the test inoculum in the immediate area of the injection was killed. At dosages of 2 to 2½ cc. per sq. ft. a complete kill of *Verticillium* was usually obtained throughout a 12-in. zone. The minimal exposure time required to kill *Verticillium* is short, no appreciable difference occurring between a 1 and 6 day period. Chloropicrin also appears to diffuse about equally well in all directions. These results as to dosage agree closely with the earlier results of Godfrey (3). Based on a 2 cc./sq. ft. minimum, 23 gal. or 322 lb. per acre would be required to control *Verticillium;* 2.5 cc./sq. ft. would require approximately 400 lb. per acre, the figure Godfrey favored for control of *Verticillium.*

TABLE 1.—*Percentages of inoculated test pieces showing viable Verticillium after exposure to 2.5 cc. of chloropicrin per sq. ft. Exposure times and injection depths compared. Data are averages of 6 repetitions, including tests with dried inoculum. Soil temperature approximately 18–20°C.; moisture 12.5–14 per cent*

	Injection depths									
	6 in.					12 in.				
Test piece depth, inches	1	6	12	18	24	1	6	12	18	24
Day's exposure										
1	25	0	0	100	100	100	0	0	100	100
2	0	0	0	100	100	50	0	0	75	100
4	0	0	0	100	100	25	0	0	0	50
6	0	0	0	100	100	0	0	0	0	25

Allyl bromide, studied earlier by Christie (1) as a possible soil fungicide, was effective here against *Verticillium* at concentrations of 2-3 cc./sq. ft., in a 7 day test period (Table 2). It diffused in lethal concentration laterally and downward, but not upward except at the higher dosages.

Commercial CBP-55 diffused principally downward in a manner similar to allyl bromide, and was effective against *Verticillium* in dosages of 4-5 cc./sq. ft.

SOIL FUMIGATION TESTS IN THE FIELD.—This post-laboratory method of evaluating chemical as fumigants has value largely in proportion to its applicability to field conditions. Though it might be expected that an effective field dosage would have to be somewhat higher than the minimum obtained from can tests, a dosage in gal./acre can be approximated by multiplying cc./sq. ft. by the factor 11.5. Approximate spacing of field injections can also be obtained from the can tests (6).

TABLE 2.—*Percentages of inoculated test pieces showing viable Verticillium after exposure to allyl bromide. Dosage varied; injection depth 3 in.; exposure time 7 days; soil temperature 11–16°C.; moisture 16.5 per cent*

Inoculum depth (in.)	Dosage cc/sq. ft. (injected 3") 1	2	3	4	5
1.5	100	25	100	25	0
3	100	25	0	0	0
6	100	0	0	0	0
9	100	0	0	0	0

Allyl bromide was applied in November 1950 to land known from previous experience to be heavily infested with *Verticillium*. The soil was a heavy loam, and plots were 88 sq. ft. in area (1/500 of an acre). Recent rains had left the soil moisture near field capacity, and rain fell shortly after application of the chemical. Dosages of 2,3,4, and 5 cc./sq. ft. were injected 3½ in. deep at 12-in. staggered centers. Because of wet weather 5 months elapsed before the results were checked. Results were determined by indexing soil samples carefully removed from 5 horizons at increments from the first 1½ in. down to 22 in. The details of this indexing method have been described earlier (7). Soil obtained thus from each horizon was put into 5 8-in. pots; each pot was planted with 10 seedling Bonny Best tomato plants. After 7 weeks a culture was made from basal stem tissues of each plant. The percentage of plants which became infected with the wilt *Verticillium* (infection index) from soil obtained from each horizon is given in Table 3. Results indicated a kill of Verticillium with dosages of 2–3 cc./sq. ft. except in the uppermost 3 in. of soil. This corroborated the results obtained from can tests as to the performance of allyl bromide in respect to both dosage and diffusion pattern.

Chloropicrin (commercial Larvacide) and chlorobromopropene (commercial CBP-55) were applied to land upon which susceptible chrysanthemums failed repeatedly from the Verticillium disease. The soil was a heavy loam. Chloropicrin was applied to plots A and B, which contained 100 and 150 sq. ft. respectively, CBP-55 to plots C and D which contained approximately the same areas. The dosage of chloropicrin was 3 cc./sq. ft. (approximately 3.07 were actually applied) injected 6 in. deep, and of CBP-55 4 cc./sq. ft. injected 3 in. deep. The higher than estimated minimal dosage of chloropicrin was applied because of the heavy soil and wet conditions. Rain fell during and after the treatments which puddled the surface soil and obviated the need of a water seal. Plots were planted with cultured *Verticillium*-free cuttings[2] of the Albatross chrysanthemum, a highly susceptible variety, plots A and D with 120, plot B with 168 and plot C with 184 plants each. Four months later the chloropicrin plots (A and B) showed 1 and 2 respectively (1.04 per cent) of plants diseased to the extent that the flowers were unfit for market; the CBP-55 plots (C and D), 43 and 10 respectively (17.4 per cent) of plants similarly affected; the nontreated check plots 56 per cent. After harvesting the flowers, cultures were made from the basal stem tissues of plants to determine what percentage was actually infected, and the location of infected plants in the plots. Each plant of the chloropicrin plots was cultured, and plants of entire rows of the CBP-55 and check plots. Culturing revealed 11 infected in plot A, 6 of which were peripheral, and 15 in plot B, 7 of which were peripheral, or an average of 9.1 per cent in the chloropicrin plots. One half of the infected plants were peripheral and the remainder tended to occur in groups, suggesting failure of the fumigant in specific areas, or local spread or introduction of the pathogen. Cultures of the 47 plants of 2 central rows of plot C (CBP-55) showed 36 (76.4 per cent) to be infected. A similar number of check plants were 100 per cent infected.

[2] Donated by Yoder Brothers, Inc., Barberton, Ohio.

TABLE 3.—*Percentage of tomato plants infected with Verticillium in soil removed from depths indicated after soil fumigation in field with allyl bromide at 4 dosages. Injection depth 3.5 in. Spacing 12-in. staggered centers*

Depth (in.)	Dosage[a] 2[23]	3[34]	4[46]	5[57]	Untreated control
0-1.5	91	82	78	72	81
1.5-3	48	21	43	46	99
3-6	0	0	0	8	90
6-12	0	0	0	0	28
12-22	0	0	0	0	6

[a] Unbracketed figure is cc./sq. ft.; bracketed figure is gal./acre.

SUMMARY

A method of evaluating the fungicidal effects of fumigants on *Verticillium* in soil is described. Twenty-one chemicals tested in at least 3 different concentrations were employed. These were injected into composted soil containing standardized *Verticillium* inoculum grown on tomato stem pieces placed at depths from 1.5 to 24 in. in 32-gal. cans. Results were based on recovery in culture of Verticillium from the test pieces and were expressed in cc. of chemical per sq. ft. of soil required to kill *Verticillium* to a depth of 12 in. Chloropicrin was effective against *Verticillium* at 2–2.5 cc./sq. ft. diffusing in lethal concentration nearly equally well in all directions. Allyl bromide and CBP-55 (55 per cent chlorobromopropene) were effective at 2–3 cc./sq. ft. and 4-5 cc. respectively, diffusing primarily downward and laterally, but not upward. The other chemicals tested were not effective against *Verticillium* in these tests. Reasonable agreement between the estimated field dosage from can tests and one that will control the disease in the field was achieved. In field tests, Verticillium wilt in chrysanthemum was reduced from 56 per cent to 1.04 per cent by a 3 cc./sq. ft. (35.3. gal./acre) application of commercial Larvacide, to 17.4 per cent by a 4 cc./sq. ft. (46 gal./acre) application of commercial CBP-55 on the basis of production of marketable flowers and on the basis of infected plants from 100 per cent to 9.1 per cent and to 76.4 by the Larvacide and CBP-55 respectively.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

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THIRAM FOR SMUT CONTROL IN ONION SET PLANTINGS[1]

R. H. Larson and J. C. Walker

Most of the better upland soils in the Racine-Kenosha area of Wisconsin are contaminated with the smut fungus (*Urocystis cepulae* Frost), and with many successive crops of onion this fungus has increased to such an extent that without the use of adequate control measures, onion sets cannot be grown profitably. In set culture about 70 lb. of seed are planted per acre as compared with 4–5 lb. per acre for bulb production. In smut-infested soil planted at the higher seed rate a thin, irregular stand occurs and low yields of sets of desirable size with a large percentage of over-size bulbs result. The standard method of smut control in set culture in this area has been the application of 1 pt. of 40 per cent commercial formaldehyde in 16 gal. of water at the rate of 1 gal. per 150 ft. of row. From a tank attached to either the tractor or the seeder, the solution is applied directly to the seed furrow ahead of the covering blades. Objections to this method of smut control have been the special equipment required for application, labor involved in hauling water and making up the solution, regulation of the flow to provide the proper amount per acre, injury to seedlings under dry conditions, reduction of effectiveness when a heavy rain follows immediately after planting, and occasional plugging of the seed spout owing to splashing of the solution.

In 1944 Newhall[2] announced a new method of control which consisted of stirring the seed with a 5 per cent solution of methyl cellulose and then coating with a given amount of thiram. Best results were secured with about 1 lb. of the fungicide pelleted on 1 lb. of seed when the bulb production rate of about 5 lb. of seed per acre was used. Experiments with thiram applied to seed sown at the 70-lb. seed rate for onion sets were begun in the Racine-Kenosha area in 1946 and continued through 1951. Preliminary reports have been published[3,4].

Table 1.—*Comparison of onion smut control with standard formaldehyde-drip treatment and with various amounts of thiram applied to the seed, 1946*

Treatment	Diseased plants (per cent)
NONE	49.8
HCHO 1 pint-16 gals.-2400 ft.	4.3
1/10 lb. thiram to 1 lb. seed (no sticker)	3.8
1/8 lb. thiram to 1 lb. seed + sticker	9.8
1/4 lb. thiram. to 1 lb. seed + sticker	2.5
LSD[a] 99:1	4.6

[a] The data from untreated plots were not included in analysis of variance.

Experimental results.—Thiram in the form of 50 per cent tetramethylthiuram disulfide (Arasan) was applied in various amounts to onion seed with or without methocel sticker. The latter was applied according to the method of Newhall[2]. Treated and untreated seeds were sown at the 70-lb. rate in random order in 4 replicate blocks on naturally infested soil. Random samples of seedlings from each treatment in each replicate were removed and sorted, and the percentage of diseased plants was determined when lesions from early infection had reached full development and before infected seedlings had begun to die and disappear.

Results of the 1946 experiment are in Table 1. With the 70-lb. seed rate the control with 1/10 lb. of thiram to 1 lb. of seed without sticker was equal to that with the standard formaldehyde treatment. When 1/8 lb. of thiram was used per pound of seed with sticker, the percentage of diseased plants was significantly higher than when the 1/10 lb. treatment without sticker was used. When the rate with sticker was increased to ¼ lb., the percentage of diseased

[1] Accepted for publication July 5, 1953.

[2] Newhall, A. G. 1944. Thiosan for onion smut control in New York. U. S. Dept. Agr., Pl. Dis. Reptr. 28: 881-882.

[3] Gorenz, A. M. and J. C. Walker. 1947. Influence of methocel sticker on the effectiveness of Arasan for onion-smut control. (Abs.) Phytopathology 37: 7-8.

[4] Larson, R. H. and J. C. Walker. 1952. Arasan for smut control in set-onion plantings. (Abs.) Phytopathology 42: 469.

TABLE 2.—*Comparison of onion smut control with standard formaldehyde drip treatment and with 1/10 lb. of thiram per pound of seed with and without sticker on basis of per cent plants diseased*

Year	Percentage of diseased plants in treatment indicated				
			Thiram		
	None	HCHO	With sticker	Without sticker	LSD[a] 99:1
1947	46.8	4.4	12.0	3.8	4.9
1948	88.4	7.3	11.6	3.3	1.1
1949	80.1	6.3	12.7	4.9	2.4
1950	80.4	6.1	8.8	3.5	1.1
1951	89.3	7.9	13.0	4.0	1.4
5-year average	77.0	6.4	11.6	3.9	

[a] Data from untreated plots were not included in analysis of variance.

plants fell to approximately the same level as with the 1/10 lb. rate without sticker. This difference was interpreted as probably owing to the fact that the sticker inactivated a certain amount of the thiram. Results indicated that with the heavy seed rate and with somewhat more than 10 times as much fungicide applied per ft. of row as with the 5-lb. seed rate, enough thiram was supplied at the rate of 1/10 lb. per pound of seed. Furthermore it was indicated that the use of thiram at this rate would be more effective without sticker than with it.

During the next 5 seasons (1947–1951), experiments were confined to comparisons of standard formaldehyde drip treatment with 1/10 lb. thiram per pound of seed with and without sticker. Results are in Table 2. In each of the 5 seasons thiram without sticker was more effective than formaldehyde; in 3 of the 5 seasons the differences in percentage of diseased seedlings were highly significant. On the contrary when sticker was used with the same amount of thiram, the percentage of diseased plants was in each season highly significantly greater than in the formaldehyde treatment.

The use of 1/10 lb. thiram without sticker per pound of seed came into general use in the Racine-Kenosha area during the course of these experiments. In 1952, 4 large commercial fields known to be heavily infested with the smut organism and sown with seed treated with thiram were surveyed for occurrence of disease. The highest percentage of diseased plants recorded was 3.6.

DISCUSSION AND SUMMARY

It is shown, as a result of 6 years of experimentation in the Racine-Kenosha area of Wisconsin, that onion smut is effectively controlled in set culture on upland soil by the application of 1/10 lb. of thiram to 1 lb. of seed without sticker when approximately 70 lb. of seed per acre are used. It is to be emphasized that the results do not apply to culture in which about 5 pounds per acre is used for large-bulb production. At the 70 pound per acre seed rate about 7 pounds of thiram is applied per acre. It is to be noted that Newhall[5] secured good control with the low seed rate on muck soil in New York when 10 pounds of tersan, or 20 pounds of a mixture of 1 part of thiram with 2 parts of pyrophyllite were applied per acre by blowing into the seed furrow with a special attachment. Newhall, Rawlins and Sloan[6] later reported that trials indicated that application of 3 pounds of thiram per acre in a dust containing an insecticide and a filler was effective.

Whether at the high seed rate for sets a smaller amount than 1/10 lb. of thiram to 1 lb. of seed is adequate has not been determined. Results reported herein show that in set production when the thiram is applied at 1/10 lb. rate, smut is controlled effectively without use of sticker and without any special equipment.

DIVISION OF VEGETABLE CROPS AND DISEASES
UNITED STATES DEPARTMENT OF AGRICULTURE
AND
DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

[5] Newhall, A. G. 1951. New methods of controlling onion smut. (Abs.) Phytopathology 41: 28.

[6] Newhall, A. G., W. A. Rawlins and M. J. Sloan. Control of onion maggot and smut by one treatment at seeding time. (Abs.) Phytopathology 42: 15.

TESTS FOR FUNGICIDAL SOLUTIONS OF FORMALDEHYDE AND OF SEVERAL MERCURIALS[1]

Dorothy Polley, V. L. Miller, C. J. Gould and W. D. Courtney

Two diseases of narcissus bulbs are controlled by treatment with nematocidal or fungicidal solutions. One of these diseases, caused by bulb and stem nematodes of the *Ditylenchus* species, is controlled by soaking the bulbs for 4 hours in warm, dilute formaldehyde (1). Miller et al. (4) have shown that in commercial tanks the formaldehyde solution may decrease in strength as much as 33 per cent in 4–7 dipping periods.

The second condition is basal rot caused by *Fusarium oxysporum f. narcissi* and is commonly controlled by dipping the bulbs in phenyl mercuric acetate solution (3). Samples of these solutions from growers' tanks were analyzed in the laboratory to show the loss of fungicide through repeated use (Table 1). Even though phenyl mercuric acetate was being added at irregular intervals, the loss was so great that it was a question whether disinfection or inoculation was occurring in the tanks. There was no constant relationship between the number of loads and the decrease in concentration. Uupublished data of the authors show that, under standardized conditions, contact with various soil types causes a loss of fungicide varying from 31 to 98 per cent.

From these observations it became apparent that some means of testing the concentrations of the dipping solutions was needed so they could be kept at a constant, optimum level. The following tests were developed for use by the bulb growers, inspectors, county agents, and others.

By a slight modification, the phenyl mercuric acetate test may be used to determine the concentration of several other important organic mercurial fungicide solutions. The commercial formulations of these closely related fungicides are 2 per cent Ceresan, New Improved Ceresan, Ceresan M, and Panogen. They are used for control of diseases of narcissus and gladiolus bulbs, and as a seed treatment for cereal grains.

FORMALDEHYDE TEST.—The recommended concentration of warm water U.S.P. formaldehyde solution for control of nematodes is .50 per cent (1 pint formalin to 25 gallons water) (2). By testing a 10 ml. sample from the tank and comparing it with a graph prepared from known amounts of formalin, one may determine the strength of a .30 to .70 per cent solution. The method is a modification of a standard chemical procedure with the following reactions:

$$Na_2SO_3 + HCHO + H_2O \longrightarrow NaOH + CH_2(NaSO_3)OH$$

$$2NaOH + H_2SO_4 \longrightarrow Na_2SO_4 + 2H_2O$$

TABLE 1.—*Analysis of growers' tank solutions*

Loads dipped	P.p.m. phenyl mercuric acetate			
	Tank 1	Tank 2	Tank 3	Tank 4
1	144	142	138	121
3	96	117	132	94
5	71	109	118	76
7	20	74	104	53
9	4	62	87	
11	8	<2	78	
13	2		68	
15	4			
17	<2			

Materials:

1. Magnesol industrial regular.[2] A 250 ml. narrow mouth bottle is a satisfactory container.
2. Thymolphthalein indicator. A 0.3 per cent solution is prepared in 50 per cent isopropanol.
3. Sodium hydroxide, 0.3 N.
4. Sodium sulfite, 1.3 M. This solution, 19.2 g. in 60 ml. water, must be made up new every week.
5. Sulfuric acid, 1 N. Seven ml. of concentrated sulfuric acid and .25 g. of Pluronic F68[2] made up to 250 ml. in distilled water.
6. Standard formaldehyde solutions. One ml. of clear U.S.P. formaldehyde without sediment is diluted to 150 ml. with water to give a .67 per cent standard solution. In like manner 1 ml. is diluted to 200 ml., and 1 ml. to 250 ml. to give .50 per cent and .40 per cent standard solutions.
7. Test tube and No. 4 rubber stopper. A tube 23 × 165 mm. is calibrated at approximately 5 and 25 ml.
8. Funnel, short stem and 65 mm. wide at top.
9. Filter paper, S. & S. No. 560, 12.5 cm. diameter.
10. Two test tubes, 16 × 150 mm.
11. Two test tubes. Tubes 150 × 19 mm. are calibrated at 10 and 12 ml.
12. Medicine dropper, at least 5 in. long.
13. Graph paper.

Solutions 2, 3 and 5 must be in tightly stoppered bottles with small tipped droppers fitted in the stoppers. All solutions must be kept stoppered when not in use.

A diagram of the arrangement of the kit which proved satisfactory in the field is shown in Fig. 1.

Procedure.—A graph of standard solutions must be prepared for each set of reagents, since the concentration of the acid or the size of the drops may vary with each set. This graph is then used for comparison until new reagent 5 is made or until the size of the drop of reagent 5 is changed by breaking the tip of the dropper

[1] Accepted for publication July 13, 1953.
Scientific Paper No. 1208, Washington Agricultural Experiment Stations, Pullman. Project No. 724.
A portion of this paper was presented at the Northwest Regional American Chemical Society Meeting in Pullman, Washington, June 13, 1953.

[2] The Magnesol was supplied by the Westvaco Chemical Division, South Charleston, W. Va., and the Pluronic F68 by the Wyandotte Chemicals Corporation, Wyandotte, Mich.

or by adhering dirt particles.

Standard solutions containing .40, .50, and .67 per cent formalin are tested by the following steps. The test tube 11 is rinsed once with the standard solution and then filled exactly to the 10 ml. mark. Any excess is removed with the medicine dropper. Ten drops of the indicator, solution 2, are added. The pH is adjusted by drops of reagent 3 until the blue color remains after swirling. The tube is filled to the second line with reagent 4 and swirled. A slight excess of reagent 4 does no harm, but a large amount is sufficient cause to discard the analysis. Reagent 5 is added dropwise with swirling until the blue color just disappears, and the number of drops required is recorded. These acid drops must be uniform in size or the test is not accurate. Therefore, extreme care must be taken to hold the dropper in a vertical position and to see that there are no air bubbles in the drops. For exact work, drops should be 2 seconds or more apart. Each standard solution is tested until duplicate results are obtained at each of the 3 levels. Using these values the standard graph is drawn (Fig. 2).

If the sample from the tank is yellow or only slightly brown it may be analyzed by the same procedure as for the standards. However, a clarification step must be added for solutions which are so dark that the blue color of the indicator in alkali is masked. This is done by rinsing tube 7 with a little of the sample, adding reagent 1 to approximately the lower mark and filling the tube to the upper mark with the sample. It is stoppered, shaken 20 times, and the solution is filtered into tube 10 which has been rinsed with some of the original sample. The cleared solution is analyzed as in the procedure for the standards. The number of drops of acid used is recorded, and the strength of the tank sample is found by comparison with the standard graph. Upon completion of each test the tubes 7, 10 and 11, and the funnel are washed with tap water to prepare them for reuse.

Discussion.—Samples were taken from commercial growers' tanks and analyzed by the methone gravimetric method as modified by Yoe and Reid (6), and by the above procedure. Data in Table 2 show that the field test gave reasonable accuracy when compared to the gravimetric procedure and that independent workers obtained comparable results.

The step necessary to clear brown colored solutions has no effect upon the concentration of formaldehyde in tank samples. Standard solutions showed no change after adding this step, and varying the amount of Magnesol used made no difference. The Pluronic F68 added to the sulfuric acid caused the formation of smaller and more uniform drops of the reagent.

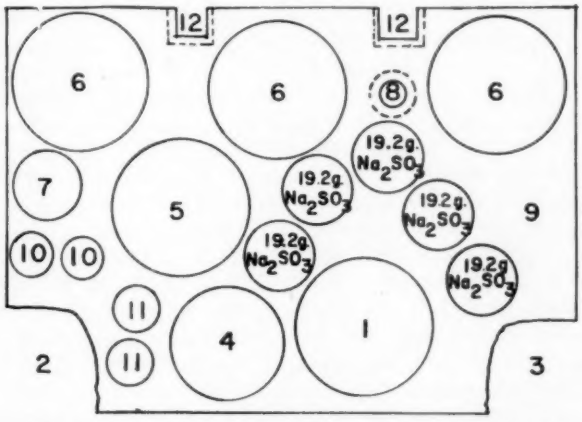

FIG. 1. Arrangement of reagents and apparatus in formaldehyde testing kit. Numbers refer to items in section entitled "Materials." Dotted lines indicate vertical support. The inside dimensions of the box are 25.3 × 17.9 × 18.2 cm.

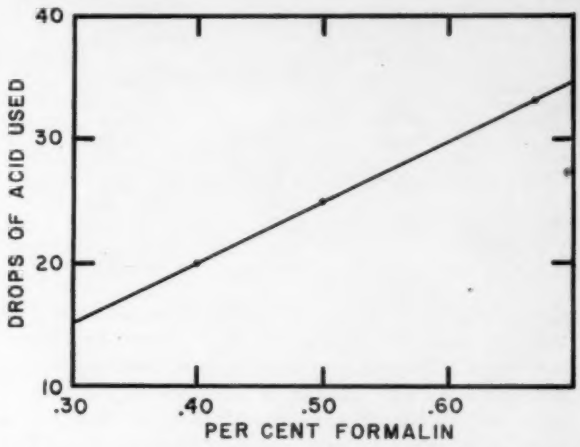

FIG. 2. A sample calibration graph for the formaldehyde test.

TABLE 2.—*Accuracy of procedure*

Gravimetric procedure	Field test by chemist	Field test by laboratory worker
per cent formalin	per cent formalin	per cent formalin
0.31	0.30	0.31
	0.32	0.32
0.39	0.37	0.38
	0.42	0.44
0.48	0.47	0.44
	0.52	0.50
0.59	0.57	0.57
	0.61	0.62

PHENYL MERCURIC ACETATE TEST.[3]—A solution of 200 p.p.m. (approximately 1 lb in 600 gal. of water) phenyl mercuric acetate is recommended as a bulb dip to prevent basal rot of narcissus (2). The concentration of the tank solution may be determined by testing a measured amount and comparing the color produced to a set of artificial permanent color standards equivalent to 80–215 p.p.m. phenyl mercuric acetate. This test is a modification of a laboratory procedure developed by the authors (5). It is based on the quantitative transfer of the phenyl mercuric acetate from the aqueous phase to a chloroform phase, and

[3] The samples of phenyl mercuric acetate used in these tests were supplied by F. W. Berk Co., Wood Ridge, N. J.

TABLE 3.—*Composition of color standards*

P.p.m. PMA represented	$Ni(NO_3)_2 \cdot 6H_2O$ 75.0% w/v	$CoCl_2 \cdot 6H_2O$ 45.4% w/v	5.0% w/v	$K_2Cr_2O_7$ 1.0% w/v	$CuSO_4 \cdot 5H_2O$ 30.0% w/v	H_2O
	ml.	ml.	ml.	ml.	ml.	ml.
80	1.65	0.45	—	0.045	3.10	5.0
110	2.50	0.80	—	0.19	3.15	11.7
140	1.00	0.82	—	0.45	3.40	14.0
170	0.75	0.61	—	0.65	1.25	18.0
200	0.15	—	2.50	0.60	—	11.8
215	—	—	2.45	0.65	—	11.0

the subsequent reaction of diphenylthiocarbazone (dithizone) with the phenyl mercuric acetate to change the green dithizone to yellow phenyl mercuric dithizonate. The color change is not specific for phenyl mercuric acetate since many metals in solution give a color with the reagent. However, by extracting the phenyl mercuric acetate with chloroform, 1000 μg. of many metals, including mercury, cause no interference. Calculations based on unpublished data show that the extraction of the phenyl mercuric acetate from the water phase to the chloroform phase is 99.5 per cent complete under the conditions of this test.

Materials:

Since this test requires several materials to be made with quantitative precision and with no contamination, it is suggested that a professional chemist prepare the reagents. Once the kit is assembled, however, the test may be readily performed by one untrained in chemical methods.

1. Artificial color standards. These standards representing 80, 110, 140, 170, 200, and 215 p.p.m. phenyl mercuric acetate under the conditions of this test are mixtures of 5 reagents in the proportions given in Table 3. Each concentrated metallic solution contains 1 drop of concentrated mineral acid to repress hydrolysis. Ten ml. of each of these mixtures are placed in a test tube 23 mm. in diameter and sealed off. In this sealed tube the standard will keep indefinitely. Another, but less satisfactory, means of keeping the standards is in screw-cap bottles of 27 mm. diameter with a protective paraffin seal.

2. Dithizone (diphenylthiocarbazone) in tubes. A solution containing exactly 83 mg. of dithizone (Eastman Kodak No. 3092) per 100 ml. is prepared in chloroform. Pieces of glass tubing approximately 40 mm. long and 8 mm. in outside diameter are sealed at one end, washed carefully with dichromate sulfuric acid mixture, and rinsed several times with distilled water. After being dried, the tubes are handled with stainless steel forceps and placed in blocks of wood which have 8 mm. × 30 mm. deep holes. Exactly 0.100 ml. of the dithizone solution is pipetted into each tube and the chloroform allowed to evaporate. A cardboard lid is placed over the tubes in the block. Tubes prepared in this manner and stored have shown no loss in dithizone over a period of 3 months.

3. Hydrochloric acid, 1.0 N.—To this is added 1 ml. of dithizone extracted 20 per cent hydroxylamine hydrochloride for each 20 ml. of acid. The extraction is made by shaking the hydrochloride with a dilute chloroform solution of dithizone in a separatory funnel and discarding the chloroform layer. This is repeated until the dithizone solution does not change color. The Baker and Adamson reagent has proved satisfactory.

4. Chloroform, ACS grade.

5. Acetic acid, 0.3 N.

6. Distilled water.

7. Cylinder. Marked at 17.5 ml. for measuring hydrochloric acid.

8. Separatory funnel, 60 ml.

9. Cylinder. A 16 mm. test tube with a pouring lip is graduated at 5 and 18.5 ml. for measuring chloroform.

10. Test tubes. Two 9 mm. tubes with pouring lips are calibrated to deliver 3.0 ml. The one to be used for measuring the sample from the tank is marked with an *S* and the one for measuring the chloroform extract is marked with a *C* so that they will not be used interchangeably.

11. Medicine dropper.

12. Erlenmeyer flask, 125 ml. glass stoppered.

13. Stainless steel forceps.

14. Cylinder. A vial is marked at the 10 ml. level for measuring acetic acid.

15. Two tubes or vials. Which ever is used in making the artificial standards should be used for these color comparison tubes.

16. Fluorescent lamp and white paper.

All the glassware is cleaned by a nitric acid wash and several rinses of distilled water.

Procedure.—Because this test is sensitive to daylight, it must be conducted in a building and the color comparisons *must* be made under a fluorescent light. Chloroform solutions of dithizone are dichoric, appearing reddish brown when viewed by tungsten light. A bulb storage room with a fluorescent desk lamp is a satisfactory arrangement.

Cylinder 7 is filled to the mark with reagent 3 and the contents poured into the separatory funnel 8. Cylinder 9 is filled exactly to the upper mark with chloroform, reagent 4, and poured carefully into the separatory funnel 8. Test tube 10 marked *S* is rinsed with some of the tank solution and then filled exactly to the mark with the solution. Any excess is removed with the extracting dropper 11, and the measured solution is poured into the funnel 8. The funnel is shaken vigorously for 1½ minutes. While the layers are separating in the funnel, one of the tubes 2 is

TABLE 4.—*Phenyl mercuric acetate addition rates to bring solution up to 200 p.p.m.*

Tank sample	PMA to add to make 200 p.p.m.
p.p.m. PMA	oz. per 100 gal. of solution
0	2.7
80	1.6
110	1.2
140	0.8
170	0.4
200	0

placed in flask 12 using the forceps to handle the tube. Cylinder 9 is filled exactly to the lower mark with chloroform and added to flask 12. All the color is dissolved from the tube by gentle swirling. The stopper is removed from funnel 8, the tube 10 marked *C* is rinsed with a little of the chloroform (lower) layer from the funnel before filling exactly to the mark with more of this same solution and is then poured into the dithizone solution in flask 12. Cylinder 14 is filled to the mark with the dilute acetic acid, reagent 5, and emptied into flask 12. The flask is tightly stoppered and the solution is mixed by shaking and vigorous swirling for ½ minute. The contents of flask 12 (except the glass tube) are then poured into the comparison tube and estimate of the phenyl mercuric acetate content made by comparing with the set of standards. This color matching is done with a white paper for a background and under a fluorescent light. After determining the strength of the tank sample the amount of phenyl mercuric acetate to add to bring the solution to the recommended concentration is found in Table 4. The funnel 8, flask 12, and tube 15, are washed with tap water followed by a distilled water rinse to prepare them for reuse.

Discussion.—Small squares of filter paper were first tried in place of the tubes for containing the measured amount of dithizone. This did not prove satisfactory because the dithizone was partially destroyed in a short time.

Using a kit prepared for this test, a commercial bulb grower determined the strength of his treating solution after every few loads. Following a demonstration and a practice run on the procedure, he had no difficulty in following the directions.

The test should be made on a sample which is taken from the tank shortly before it is to be used, since the concentration may change on standing. Samples which stood for 5 days after being in contact with soil showed a variable loss of concentration.

Table 5 shows the accuracy with which the test was performed by persons with varying knowledge of chemical methods. The percentage of the results which were within 10 p.p.m. of the standard procedure value was from 75 per cent for the person with no chemical training to 100 per cent for the chemist, and the maximum deviation was 25 p.p.m. Accuracy may be improved by running duplicate samples.

This test is not specific for phenyl mercuric acetate

TABLE 5.—*Accuracy of procedure for phenyl mercuric acetate*

Standard laboratory procedure	Field test by		
	chemist	laboratory worker	field worker
p.p.m. PMA	p.p.m. PMA	p.p.m. PMA	p.p.m. PMA
74	<80	<80	<80
80		80	94
95	90	90	115
101		110	110
119	115	110	100
126	120	120	120
128		120	130
139		135	135
159		135	150
163	155	145	160
168	165	160	175
172	165	165	160
188		180	180
190	195	180	185
207	205	210	210
210		210	210

but rather a test for phenyl, ethyl, methyl, and possibly other organic mercuric compounds. Therefore, by adjusting the volume of sample this test will determine the ethyl mercuric phosphate[4], ethyl mercuric chloride, ethyl mercuric p-toluene sulfonanilide, methyl mercuric dicyan diamide[4], or phenyl mercuric acetate fungicide in any desired range of concentration.

In the above procedure a 3 ml. sample of solution containing 80–215 p.p.m. or a total of 240–645 μg. of phenyl mercuric acetate gives the green to yellow colors of the artificial standard. It requires 200–542 μg. of ethyl mercuric phosphate to give the same color range. The growers of gladiolus who use a 120 p.p.m. dip of the ethyl compound could modify the above procedure by taking a 4 ml. tank sample instead of the 3 ml. sample used in the directions. Their testing range for the same artificial standards would then be 50 to 135 p.p.m. Table 6 gives the total μg. of the active ingredients which will compare to each of the artificial standards.

To test for the methyl mercuric compound in Panogen, a modification besides the change in volume is necessary. The active ingredient is more soluble in 3 NHCl than in sodium chloride; therefore, reagent 3 is changed so that it is 1 N in respect to sodium chloride, .05 N in hydrochloric acid, and contains 1 ml. of the 20 per cent hydroxylamine hydrochloride for each 400 ml. of the solution. The formulation also contains a red dye which interferes by masking the color reaction at the low pH of the regular procedure. The dye was found to be red under acid conditions but became colorless at a pH of 5.5 or above. A pH 6.0 buffer used in place of reagent 5 gave the higher pH required to remove the interfering color and still contained some

[4] The ethyl mercuric compounds for these tests were supplied by E. I. du Pont de Nemours and Co., and the methyl mercuric dicyan diamide by Panogen, Inc., New York.

TABLE 6.—*Total amount of organic mercury compounds represented by the artificial color standards*

Artificial color standard	Phenyl mercuric acetate [a]	Ethyl mercuric chloride [b]	Ethyl mercuric phospate [c]	Ethyl mercuric p-toluene sulfonanilide [d]	Methyl mercuric dicyan diamide [e]
	μg.	μg.	μg.	μg.	μg.
80	240	203	200	363	240
110	330	280	276	501	330
140	420	356	352	639	420
170	510	433	428	777	510
200	600	510	504	915	600
215	645 or more	549 or more	542 or more	984 or more	645 or more

[a] Mersolite 8 contains 97.5 per cent phenyl mercuric acetate.
[b] 2% Ceresan contains 2 per cent ethyl mercuric chloride.
[c] New Improved Ceresan contains 5 per cent ethyl mercuric phosphate.
[d] Ceresan M contains 7.7 per cent ethyl mercuric p-toluene sulfonanilide.
[e] Panogen contains 2.2 per cent methyl mercuric dicyan diamide.

acetic acid which is believed necessary to stabilize the dithizonate color. The pH 6.0 buffer contains 77 gm. of ammonium acetate trihydrate per liter and sufficient acetic acid to give the required pH value.

Table 7 shows the accuracy of this test for the ethyl and methyl mercuric compounds is comparable to the accuracy for phenyl mercuric acetate. A difference of 10 p.p.m. of the phenyl compound is equivalent to 8 p.p.m. ethyl mercuric chloride or phosphate, 15 p.p.m. ethyl mercuric p-toluene sulfonanilide, and 10 p.p.m. methyl mercuric dicyan diamide.

SUMMARY

Two tests are presented for the use of bulb growers to determine the strength of formaldehyde or of phenyl mercuric acetate in their dipping solutions. By slight modification of the latter test it may be used to determine the concentration of methyl mercuric and several ethyl mercuric fungicides. From the procedure and reagents described, a self-contained testing kit may be prepared. The accuracy of the tests is satisfactory for use under field conditions.

STATE COLLEGE OF WASHINGTON
AND
U. S. DEPARTMENT OF AGRICULTURE
DIVISION OF NEMATOLOGY
WESTERN WASHINGTON EXPERIMENT STATION
PUYALLUP, WASHINGTON

TABLE 7.—*Accuracy of procedure for ethyl and methyl mercuric compounds*

Ethyl mercuric chloride		Ethyl mercuric phosphate		Ethyl mercuric p-toluene sulfonanilide		Methyl mercuric dicyan diamide	
Standard procedure	Field test	Standard procedure	Field test	Standard procedure	Field test	Standard procedure	Field test
p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
62	70	72	75	136	144	88	90
74	76	85	92	160	175	110	115
76	81	113	109	207	205	155	150
114	119	145	147	207	198	179	180

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A CYST-FORMING NEMATODE ATTACKING CLOVERS IN ILLINOIS[1]

J. W. Gerdemann and M. B. Linford

During a study of clover root rots in 1950, cysts and enlarged female nematodes of a species of *Heterodera* were found attached to roots of red clover, *Trifolium pratense* L. The plants were from a greenhouse bench of nonsterile soil obtained from a pasture in Champaign County, Illinois. In that pasture the nematode was found associated with white clover, *T. repens* L. Incomplete surveys have found it distributed widely but sparingly from the latitude of Chicago to the southern end of the state, occurring in pastures, lawns, and roadsides chiefly in association with white clover. In fields of red clover, only a few very light infestations have been detected. The distribution pattern suggests that this nematode is not a recent introduction.

Details of life history, pathogenicity, and host range are reported here together with the basis for identifying this nematode as *Heterodera schachtii* var. *trifolii* Goffart, the clover-root nematode. These investigations have utilized only nematodes from Champaign County, Illinois, chiefly from subcultures from the original greenhouse population.

Since 1932, when the clover-root nematode was reported by Goffart (5) in Schleswig-Holstein, it has been found in the Netherlands (9) and Britain (1) and recently has been reported from 2 localities in British Columbia (7) and 1 in California (11). What may have been the same nematode, despite reports of very different pathological effects, was described from Utah in 1938 (8).

THE NEMATODE.—No males have been found while examining numerous greenhouse cultures established from selected cysts. This agrees with the experience of Raski and Hart (11) although Franklin (4) and McBeth (8) described males.

The lemon-shaped females and brown cysts (Figs. 1, A-C) are indistinguishable from those described from clovers by other investigators (1, 4, 6, 8, 11); and, except for a transitory yellow color as the white females change into brown cysts, they are like those of typical *Heterodera schachtii* Schmidt from sugar beet. The larger females and especially the cysts are so weakly attached to clover roots that many fall away while roots are being prepared for examination. Even such roots as shown in Figure 1, B can appear lightly infested if washed without the greatest care. Plump, full cysts may be recovered from soil, along with variable quantities of debris, by wet sifting and decanting without the prior desiccation required by flotation methods. The largest can be washed through the 0.71 mm. openings of a No. 25 sieve, and even the smallest are retained by the 0.25 mm. openings of the No. 60.

Three characteristics of females and cysts are not always apparent and may be overlooked. The females deposit variable numbers of eggs in a gelatinous matrix that comes to envelop the posterior part of the body; but these eggs may soon hatch and the matrix itself usually is dislodged and lost during removal of a sample from soil. The "subcrystalline layer," a whitish coating over part or most of the body, may often be seen about the time females turn brown, but this too is readily lost. The yellow color phase reported (4, 11) as a point of distinction from the sugar-beet nematode, occurs in the Illinois material although sometimes it is difficult to find and not all cysts pass through it. Some females change directly from white to a very pale brown that darkens gradually; others first develop irregular brown patches that enlarge to involve the entire body.

Despite great variability of cyst size that makes it difficult to characterize a population adequately, measurements are reported here for comparison with those summarized by Franklin (4). These appear to be the first such figures for North American material. Lengths measured from calibrated camera lucida tracings are presented both as total length including neck and vulva and as length with neck omitted for comparison with Franklin's measurements. Only full cysts that had not been desiccated were measured.

Twenty-two cysts from white clover in a pasture, collected during January, were characterized as follows: total length 430–1040 μ, mean 773μ; length without neck 420–950 μ, mean 725 μ; breadth 270–610 μ, mean 469 μ; ratio total length divided by breadth 1.3–2.1, mean 1.6; ratio without neck 1.2–1.9, mean 1.5.

Twenty-three cysts from a greenhouse culture of Ladino clover averaged slightly smaller and more slender: total length 535–1065 μ, mean 742 μ; length without neck 520–1000 μ, mean 678 μ; breadth 250–540 μ, mean 391 μ; ratio with neck 1.5–2.4, mean 1.9; ratio without neck 1.2–2.0, mean 1.7.

The smaller mean cyst length exceeds that reported by Goffart (6) by 17 per cent and, without neck, the mean exceeds the larger of Franklin's samples by 21 per cent. In length-breadth ratios, however, the Illinois samples agree well with the published ratios of 1.7 with neck and 1.5 without.

Larvae for measurement were removed from infested soil by wet sifting and decanting, killed with gentle heat, and drawn to scale with a calibrated camera lucida. Lengths of apparently normal larvae in several samples ranged between 475 μ and 585 μ. The mean of 28 larvae from a warm greenhouse culture of Ladino clover was 527 μ; the mean of 15 collected early in March from white clover in a lawn, was 537 μ. Means for various clover populations reported by other workers are: 480 μ, 481 μ, 491 μ, 502 μ, 514 μ, 517 μ, and

[1] Accepted for publication July 8, 1953.
Approved for publication by the Director of the Illinois Agricultural Experiment Station.

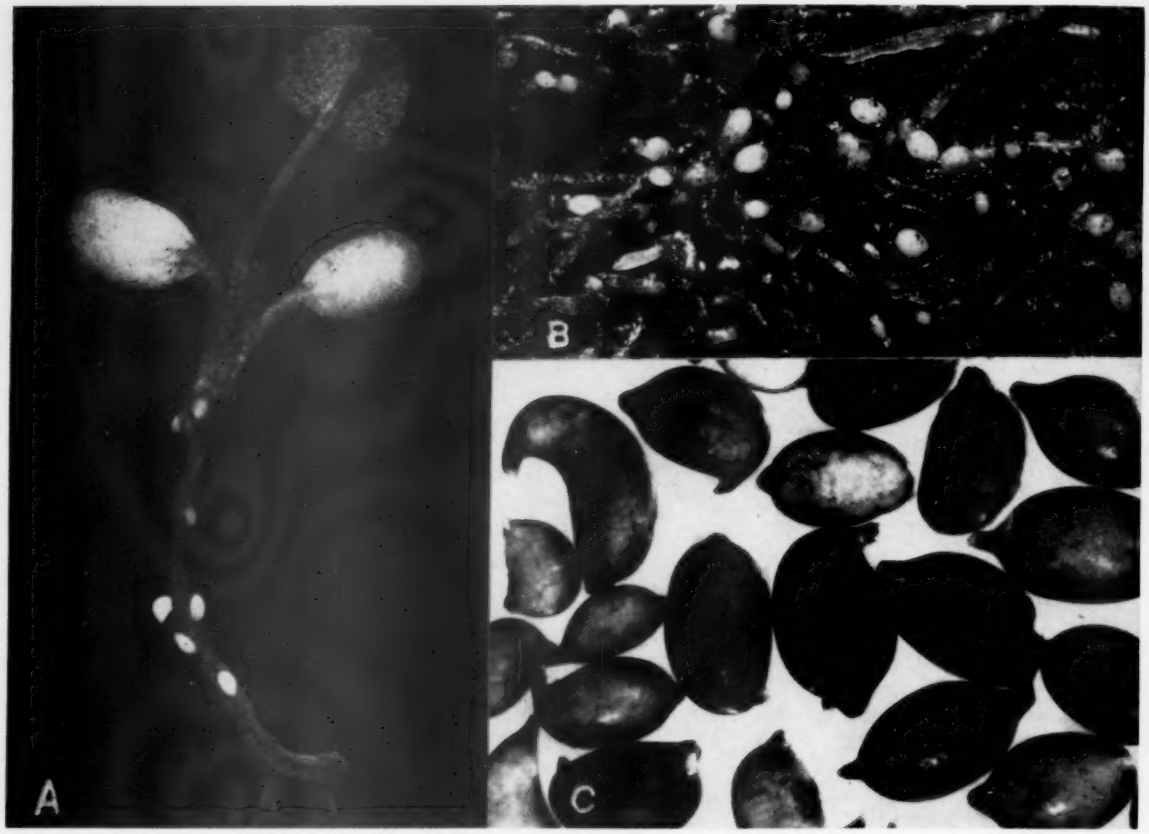

FIG. 1. A) White clover seedling severely damaged by *Heterodera schachtii*. var. *trifolii*, showing enlarged white females attached to the root and breaking through the cortex of the hypocotyl, and also showing the tap-root and 2 laterals "blinded" by necrosis and disintegration of apical parts, ×7.5. B) White females attached to roots of Ladino clover grown against a pane of glass. Brown cysts were present but are not seen here against the dark soil background, ×8. C) Representative cysts containing eggs, ×30.

529 μ. Only the last mean, reported by Franklin (2) in 1940 but not cited by her in 1951 (4), falls between the 2 Illinois means.

Larval stylet length varied from 25 μ to 32 μ with a mean of 28.5 μ compared with published figures of 24 μ, 25–27 μ, and 26–29 μ, the last figures being those of Raski and Hart (11). Distance from stylet base to dorsal gland duct orifice varied little from 6 μ. Ratio of body length to greatest diameter averaged 25.4.

LIFE HISTORY.—Goffart (6) concluded that the clover-root nematode develops 2 generations per year in Schleswig-Holstein, forming cysts in July and again in October. Observation of naturally occurring infestations in Illinois, however, indicated no limited periods of cyst formation. Soil with white clover roots, collected in January from below a crust of frozen soil, contained free larvae as well as cysts still attached to roots. More ample collections in early March, when clover top growth was just resuming, included all stages from free larvae to cysts. This has been true generally of samples collected later in the spring and during the summer. Apparently, in establishing stands of its hardy perennial clover host, this nematode develops in overlapping generations throughout the year, although undoubtedly it is greatly slowed in winter.

The minimum time required for 1 generation to develop was determined under greenhouse conditions where air and soil temperatures varied between 15° and 27° C. Seedlings of Ladino clover with primary roots 3–7 cm. long and with secondary roots started, were transplanted into an artificially infested sand-soil mixture to allow larvae to enter the roots. After 24 hours the plants were washed out of this soil, rinsed to remove nematodes that had not entered the roots, and transplanted in groups of 3 into pots of non-infested sand. To permit study of early stages of entry and establishment of the parasites, 3 or more plants were washed out every 24 hours for the first 10 days, and their roots cleared and stained in lactophenol with acid fuchsin. Other groups of plants were treated similarly after longer intervals until the nematodes had matured and produced progeny, and the new generation of nematodes had established within the roots.

In this test as in others, most of the larvae entered roots through the immature zone behind the root tip, as reported by McBeth (8), but they also seemed able to enter any part of the root that had its primary cor-

tex intact. They even entered hypocotyls, cotyledons, and leaves when these parts were covered with moist infested soil.

The rate of development observed here was similar to that found by Raski (10) for *Heterodera schachtii* in sugar beets. First eggs were seen within a few females 28 days after larvae entered the roots. After 31 days, some females were more than half full of eggs. Small gelatinous matrices were observed covering the vulva after 28 days, but the beginning of egg laying was not observed. Too many of the larger matrices were dislodged and lost in preparation for microscopic examination. Fifty days after first infection, large numbers of larvae from the new generation had already entered new roots, and the most advanced had developed to the size attained in 10 days by first-generation larvae. This indicated a minimum generation time of approximately 40 days. After 77 days, hatching had begun from eggs produced by the second generation.

After 50 and 53 days, when the new generation was well established, most of the first-generation females were still white. When placed individually in dishes of charcoal-treated water, some were still depositing eggs; these eggs hatched promptly. One female, for example, deposited 31 eggs during 48 hours; 17 of them hatched during this period. Among eggs thus deposited, some contained first-stage larvae, some contained molting individuals, and others contained the second-stage larvae which constitute the infective stage of this nematode.

At this same time, larvae had already begun to emerge from some brown cysts. During 48 hours, 35 larvae emerged from one and 91 from another. One brown cyst, from which none emerged during 48 hours in water, contained many active larvae that had already hatched and were actively thrusting their stylets against the cyst wall in the neck region and in the vulvar prominence. During the next day, 41 larvae emerged through a small hole opened in the head region of this cyst.

Despite this early emergence from cysts, it appears probable that most of the larvae that had already entered new roots had come from eggs deposited in gelatinous matrices by living females, for such matrices contained many empty egg membranes. Five of the larger matrices collected after 50–53 days were macerated individually in 0.2 per cent sodium hypochlorite. This was weaker than the solution used by Tyler (13) but it was adequate to allow eggs and empty membranes to separate, and the latter to expand from their crushed condition, making it possible to recognize and count them. Numbers of eggs and empty membranes combined ranged from 171 to 299, with a mean of 228. Numbers of empty membranes, indicating larvae already hatched, averaged 180 per matrix. Such numbers are sufficient to permit rapid multiplication of the nematode even if hatching from cysts were to be long delayed.

That the brown cyst constitutes a drought-tolerant stage in this nematode, as in other species of *Heterodera*, was confirmed when larvae emerged from cysts that had been stored 11 months in air-dried soil. However, the maximum time that eggs or larvae may remain alive within such cysts in moist soil and in association with clover roots has not been determined. Obviously, not all escape promptly from all cysts. From what is known concerning related nematodes, it is likely that some emergence may be delayed several weeks or even months, much longer than the minimum generation time. Therefore, no definition of a maximum generation time is possible. Once this nematode has established itself in a perennial host, one would expect hatching and release of larvae to occur at any time that temperature and soil moisture are favorable but, in the absence of information concerning the influence of environment upon rate of development, there is no basis on which to estimate the number of generations per year. It seems certain, however, that this number must far exceed Goffart's estimate of 2.

HOST RANGE.—Plants tested as possible hosts of the clover-root nematode included important crops of this region and also plants known to be hosts of forms of *Heterodera* with which this nematode might be confused. Several plantings were made in the greenhouse bench of naturally infested soil where this nematode was first found and where it was associated with *Meloidogyne hapla* Chitwood and several other plant-parasitic nematodes of lesser importance. Other tests were in a sandy soil that had been steamed under pressure and infested with cysts selected from the first source, then planted with clovers until heavy infestations of the *Heterodera* had developed. Both types of tests are summarized in Table 1, where the plants are arranged in 4 groups according to their apparent suitability to the nematode.

Group 1 includes plants in which the nematode developed very freely, producing cysts that are regarded as typical of this nematode. Some of these plants also were sensitive to nematode injury, with the result that very heavily infected rootlets died early and disintegrated. Other rootlets died before the attached females had attained full size, resulting in production of small cysts. Heavy infections that killed the roots often killed seedlings of white clover and alsike, and severely stunted Ladino clover. On curled dock and spinach, however, cysts were very abundant but injury was less severe.

Plants of Group 2 were less favorable to the nematode and less damaged by it. Individual plants of red clover and lespedeza were much more severely attacked than others, indicating a range of susceptibility within a species. Cysts developed well on peas and beans when grown in heavily infested soil, but they included many small ones distributed among the large on rootlets that remained alive. The thick cortex of the larger roots of these plants may entirely enclose mature cysts instead of allowing them to break through to the root surface, making it possible for an observer to overlook them.

Plants in Group 3 were highly resistant and some

TABLE 1.—*Relative susceptibility of tested species to Heterodera schachtii var. trifolii Goffart*

Plants tested		Variety	Naturally infested soil [a]	Artificially infested soil [b]
GROUP 1				
White clover	*Trifolium repens* L.	.....	+++ [c]	+++
Ladino clover	*T. repens* L.	.....	+++	+++
Alsike	*T. hybridum* L.	.....	+++	+++
Curled dock	*Rumex crispus* L.	.....	+++	+++
Spinach	*Spinacia oleracea* L.	Giant thick leaved	+++	+++
GROUP 2				
Red clover	*T. pratense* L.	.....	++	++
Lespedeza	*Lespedeza stipulacea* Maxim.	.....	++	++
Green bean	*Phaseolus vulgaris* L.	Stringless green pod	++	++
Garden pea	*Pisum sativum* L.	Oracle; Perfection	.....	++
Carnation	*Dianthus caryophyllus* L.	Tom Knipe; William Sim	.....	++
GROUP 3				
White sweet clover	*Melilotus alba* Desr.	Willamette & common	+	+
Yellow sweet clover	*M. officinalis* (L.) Lam.	.....	+	.....
Sugar beet	*Beta vulgaris* L.	U.S. 22/3	—	+
Garden beet	*B. vulgaris* L.	Detroit dark red	—	+
Crimson clover	*T. incarnatum* L.	Auburn	+	—
Birdsfoot trefoil	*Lotus corniculatus* L.	N. Y. strain	+	—
GROUP 4				
Alfalfa	*Medicago sativa* L.	Common Colorado	—	—
Soybean	*Glycine max* (L.) Merrill	Hawkeye	—	—
Tomato	*Lycopersicon esculentum* Mill.	Bonny Best	—	.....
Cabbage	*Brassica oleracea* L.	Copenhagen mkt.	—	.....
Wild mustard	*B. arvense* (L.) Rabenh.	.....	—	.....
Carrot	*Daucus carota* L.	Danvers half long	—	.....
Oats	*Avena sativa* L.	Clinton	—	.....
Corn	*Zea mays* L.	U. S. 13	—	.....

[a] Soil contained other nematode species.

[b] Heavy population built up from selected cysts in autoclaved sandy soil.

[c] +++ Highly susceptible, mature cysts abundant.
++ Moderately susceptible, mature cysts moderately abundant.
+ Resistant, mature cysts with eggs rare.
— Highly resistant or immune—no cysts found.

were inconsistent in reaction. Only a few small cysts were found on roots of white and yellow sweet clover. In naturally infested soil, a few cysts were found on the roots of a few of the many crimson clover and birdsfoot trefoil plants examined. Later, when tested in artificially infested soil, no cyst could be found on either species. No cysts were found on roots of either sugar beet or garden beet when grown in naturally infested soil, but a few cysts containing eggs were found on both plants when tested in artificially infested soil.

No cysts were found on roots of any of the plants listed in Group 4, although it was ascertained that larvae entered the roots of alfalfa and soybeans.

In general these host range results are similar to those reported by other investigators for *Heterodera* populations found attacking clovers. White clover, red clover, and dock have been reported susceptible by all who have tested them (3, 6, 7, 9, 11). Spinach, which is susceptible to the sugar-beet nematode, and lespedeza have not previously been reported as hosts of the clover nematode. Alsike, which was highly susceptible to the Illinois nematode, was reported susceptible by Hastings and Bosher (7) but not by Raski and Hart (11). Beans have been reported susceptible by most workers (6, 7, 9, 11); however, Franklin's results with beans were negative (4). Peas were reported susceptible by Franklin (3), Hastings and Bosher (7) and Raski and Hart (11), while negative results were reported from Europe by Goffart (6) and Oostenbrink (9). Carnations were reported susceptible by Raski and Hart (11).

The slightly susceptible plants listed in Group 3 never had many mature cysts and frequently it was necessary to examine many plants before a cyst containing eggs could be found. Results with these species were not consistent from one experiment to another. It is, therefore, not surprising that various workers have reported different results with members of this group.

Although the host range recorded here includes some hosts of the typical *Heterodera schachtii* from sugar beet, the clover-root nematode is distinguished by its ability to thrive in the clovers, its very poor development in beets, and its failure to mature in cabbage and mustard.

DISCUSSION.—Morphologically the clover-root nematode in Illinois is very similar to the *Heterodera* reported elsewhere from clover. Dimensions of larvae and cysts of the Illinois population average larger than reported by others, but differences between pop-

ulation means are not great in proportion to variability within a population. Too little is known of the effects of different methods of collection and preparation of nematodes upon apparent mean dimensions, and of the effects of environment under which nematodes develop upon their actual size, to attach much importance to the apparently greater size of this nematode in Illinois.

The host range of this nematode likewise differs from the published reports of others in minor details. Former workers generally have not specified what crop varieties they tested and the apparent difference in susceptibility among plants of red clover and lespedeza in the Illinois studies suggests that important differences may be found between cultivated varieties of 1 species. Different investigators have worked under varied but unspecified environmental conditions, and nothing is yet known about effects of environment upon susceptibility of any test plant. It therefore seems unwise to attach much significance to reports of minor differences in host range.

It seems probable, therefore, that the Illinois nematode is similar to the cyst-forming nematodes reported from clovers in other parts of the world, with the possible exception of the report from Salt Lake City (8). If only *Heterodera* were present in roots that developed gall-like swellings, then that nematode must be distinct, physiologically at least, from the clover-root nematode studied elsewhere.

Absence of males from the Illinois and California populations may prove to be an important distinction, since species of *Heterodera* generally are bisexual. It remains to be determined, however, whether this is uniformly characteristic of certain populations or whether development of males may be conditioned by some environmental conditions different from those to which these populations have been subjected.

Steiner et al (12) and Raski and Hart (11) have followed Oostenbrink (9) in regarding the clover-root nematode as a distinct species, *Heterodera trifolii* (Goffart) Oostenbrink. Differences thus far found between it and the sugar-beet nematode include the yellow color phase, questionable differences in some dimensions, and great differences in host range. Although there is no doubt that these nematodes are distinct biologically, the known differences do not appear to the writers to be of specific rank. Perhaps some later investigator, on the basis of detailed comparisons, will find consistent morphological characteristics to separate these nematodes into distinct species; but until such differences are found, the clover-root nematode seems best designated as *Heterodera schachtii* var. *trifolii* Goffart.

Goffart (6) suspected but did not entirely prove that the clover-root nematode weakened the clover plant, while Franklin (1) stated that the clover plant does not appear to be harmed by it. Raski and Hart (11) first found this nematode in California associated with poor growth of white clover in a lawn and, after reporting host range tests, noted that it appeared to be very destructive on clover. In Illinois, no naturally occurring infestation has been seen that afforded clear evidence of depression of clover growth, although some stands of infested white clover have been sparse and weak from undetermined causes. In the greenhouse, however, both in naturally infested soil and in soil first steamed and then infested with selected cysts, seedlings of white clover, Ladino clover, and alsike have been severely damaged or even killed by infestations of this nematode that built up during growth of clovers. On the basis of such observations, it seems probable that some of the infestations in Illinois lawns and pastures are limiting the growth of white and Ladino clovers. However, no infestation yet found in a red clover field has been sufficient to harm this crop appreciably.

SUMMARY

A cyst-forming nematode identified as *Heterodera schachtii* var. *trifolii* Goffart has been found widely distributed in Illinois in association with *Trifolium repens* L. in old pastures, lawns, and roadsides. It probably is not a recent introduction.

Cysts and larvae average slightly larger than reported for *Heterodera* from clover elsewhere but otherwise are very similar. Males have not been found.

In established stands of white clover, this nematode develops in overlapping generations throughout the year. In the greenhouse its minimum generation time is approximately 40 days.

In greenhouse tests, the most favorable host plants were white clover, Ladino clover, alsike, curled dock, and spinach. The 3 clovers were severely injured or sometimes killed by heavy infections. Red clover, lespedeza, green bean, garden pea and carnation were less suitable as hosts and were less injured. White sweet clover, yellow sweet clover, sugar beet, garden beet, crimson clover, and birdsfoot trefoil were highly resistant; however, a few cysts with eggs developed on these crops in some tests. No cysts were found on alfalfa, soybean, tomato, cabbage, wild mustard, carrot, oats, or corn.

It is probable that existing infestations are limiting growth of white clover and Ladino clover in some Illinois lawns and pastures.

Departments of Agronomy and Horticulture
University of Illinois
Urbana, Illinois

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PYCNIDIAL STAGE OF CHARCOAL ROT INCITING FUNGUS WITH A DISCUSSION ON ITS NOMENCLATURE [1]

M. J. Thirumalachar [2]

Charcoal rot disease of plants incited by *Macrophomina phaseoli* (Maubl.) Ashby occurs widely in many countries, causing damage to plants of economic importance. In India, charcoal rot disease damages potatoes, cotton, and jute when conditions are favorable for disease incitement. Charcoal rot disease also occurs in Bihar on other crops such as sorghum, maize, garlic, castor, sunhemp, finger millet (*Eleusine coracana* Gaertn.), and *Solanum nigrum* L. Gray discoloration followed by blackening of the stems, which bear numerous sclerotia of the fungus, are characteristic symptoms of the disease. In the case of potatoes, infection symptoms on tubers, first appear as blackening of lenticels, followed by rotting of the whole tuber during storage periods. Charcoal rot of potato in Bihar, with the control measures, has already been described by the writer (7). In the present paper the structure and development of the pycnidial stage with reference to the nomenclature of the charcoal-rot-inciting fungus are presented.

Isolations of the fungus were made from sclerotia occurring on charcoal-rot-affected tissues of jute, potato, maize stalks, garlic, roots of castor, and *Solanum nigrum*. The fungus was also isolated by transferring single pycnospores from charcoal-rot-affected kernels of maize (Fig. 2), stems of potato, Jute, and sun-hemp. The cultures were grown on potato-dextrose agar and incubated at 28°C. The fungus grew very rapidly at this temperature, producing abundant fumaceous hyphae.

As stated previously (7) the cultures from different hosts fall into 2 main types, resembling the sub-species *typica* and *sesamica* designated by Reichert and Hellinger (6), respectively. In the type resembling *typica* the hyphae are at first white, gradually turning smoky gray. Following the formation of sclerotia the mycelium breaks down. The tiny black sclerotia are uniformly distributed on the media (Fig. 3). In the type resembling *sesamica* the hyphae are at first white and gradually turn smoky gray showing concentric zonations of growth (Fig. 1). The sclerotia are few and irregularly grouped. The hyphae are not evanescent as in the former type, but remain persistent. This second type has been isolated from potato, maize kernels, and castor.

Inoculation experiments indicated that all the isolates, when inoculated into potato tuber, produce the characteristic charcoal rot symptoms. For this purpose, young vigorously growing colonies of the culture (48 hours old) incubated at 28°C were used to inoculate surface-sterilized potato tubers (Darjeeling Red Round variety). The tuber surface was wounded with a sterile needle and the young hyphae taken from the margin of the colonies were inserted. The inoculated point was sealed off with paraffin wax. The inoculated tubers were incubated at 28°C and observed for disease symptoms. After 3 days, observations indicated that all the isolates from jute, garlic, castor, sun-hemp, potato, maize, and *Solanum nigrum* produced charcoal rot symptoms in the inoculated potato within 4 days. Relatively old cultures in which sclerotial development had already started, failed to bring about infection when used for inoculation. It is therefore essential to take into consideration the age of the culture, etc., in differentiating pathogenic and non-pathogenic strains of this fungus.

According to our present knowledge of the charcoal-rot-inciting fungus, the pycnidial stages are found on diseased stems of legumes and jute, whereas on other hosts only the sclerotial stage occurs. Young (8) reported the occurrence of the pycnidial stage on stems of potato and watermelon. In the present study, the pycnidial stage occurring naturally on the hosts has been found on stems of potato, jute, sun-hemp, and kernels of maize. The pycnidia and pycnospores on

[1] Accepted for publication July 13, 1953.
[2] The writer wishes to acknowledge his indebtedness to Dr. S. Ramanujam, Director, Central Potato Research Institute, for the valuable guidance, suggestions and encouragement.

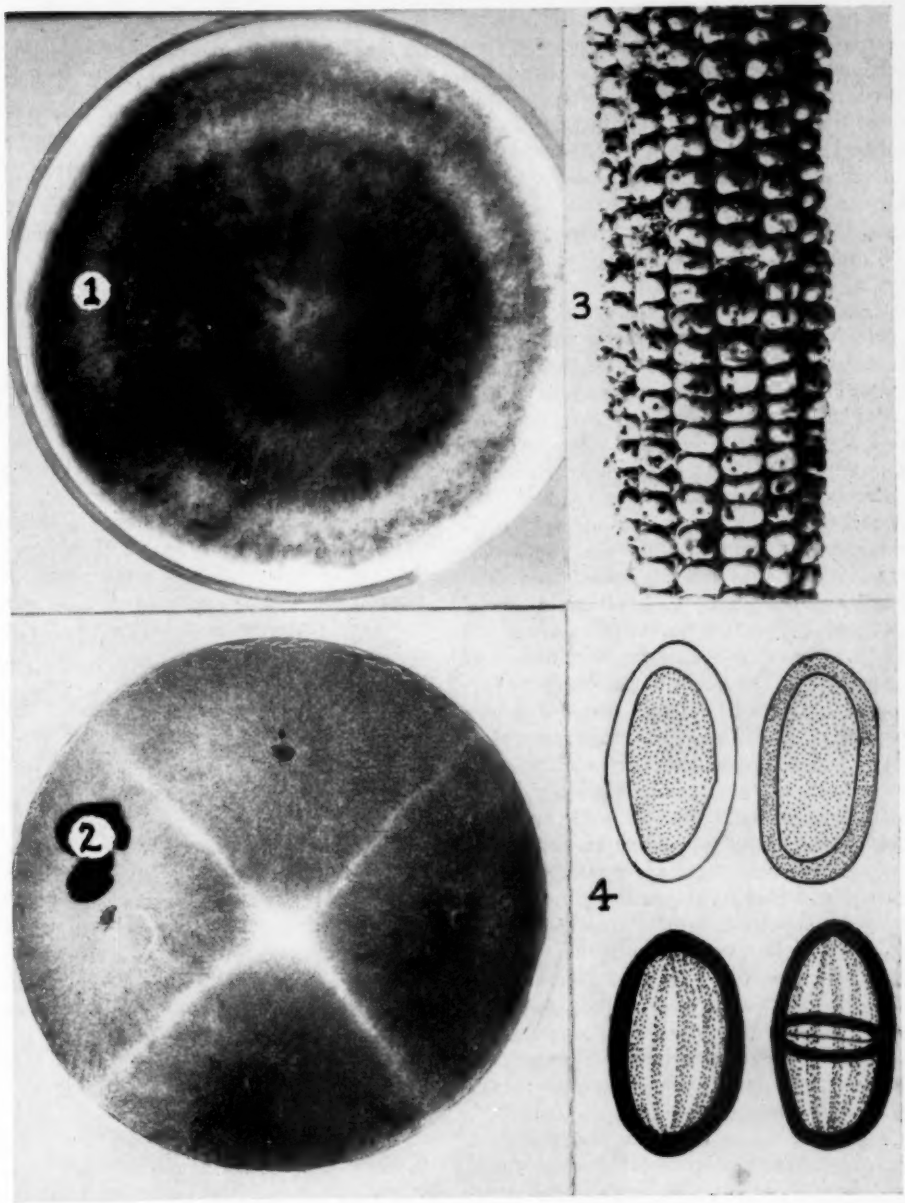

FIG. 1. Botryodiplodia phaseoli, cultural type resembling sub-species *sesamica*.
FIG. 2. *B. phaseoli*, cultural type with sclerotia resembling sub-species *typica*, showing sclerotia.
FIG. 3. Charcoal-rot-affected ear of maize showing the pycnidial pustules.
FIG. 4. Stages in the transformation of 1-celled hyaline spores to reddish-brown 2-celled condition × 500.

different hosts were morphologically identical.

During the course of the inoculation studies, it became evident that the isolates of the fungus from different hosts, when inoculated on the stem of potato plants and incubated at temperatures between 28 and 35°C, produced the pycnidial stage within a week. Inoculation experiments were conducted during April and May in Bihar, when the day temperature in the shade varied between 25 and 35°C. Young vigorously growing cultures of the fungus were used to inoculate surface sterilized stems of potted potato plants (Darjeeling Red Round) after wounding with a sterile needle. The inoculated point was sealed off with paraffin wax immediately. The first symptoms of successful inoculation became evident after 3 days, when water-soaked discoloration appeared on either side of the inoculated point. The pith tissue in the inoculated portion of the stem collapsed, leaving a hollow center. The pycnidial stage, with few sclerotia, developed on the discolored portion after 8 days, appearing as tiny black pin points. The pycnidial stage developed on inoculated potato stems in all the iso-

lates from jute, potato, sun-hemp, garlic, castor, maize, and *Solanum nigrum*. It is therefore apparent that the development or nondevelopment of the pycnidial stage is not a characteristic of specific importance, but is determined by the availability of suitable nutritional and environmental conditions. It is probable that all the isolates of the charcoal rot fungus recorded on as many as 284 hosts by Young (8), when inoculated on potato stem and incubated under suitable temperature conditions (28 and 35°C) may produce the pycnidial stage.

Development of the pycnidial stage in artificial culture has been observed in 1 of the isolates from maize. The culture showed persistent fumaceous hyphae developing in concentric zonations as in the sub-species *sesamica*. The pycnidia developed as black bodies on the superficial hyphae. The pycnidia and pycnospores were similar to those produced naturally on the host. Previously only Luttrell (3) had reported the production of pycnospores in artificial culture of the fungus.

Developmental stages of the fungus indicated that pycnidial initials appear as hyphal knots and can be easily distinguished from sclerotia. Hoffmaster et al (2) suggested that the sclerotial stages may represent undeveloped pycnidia. The present studies however, do not support this view. Mature pycnidia are erumpent, subglobose, 100-250 μ. in diameter, opening by an ostiole. The conidiophores are simple, and the pycnospores are large, ovate-ellipsoid, 18–35 $\times$ 8–12 μ, hyaline and having glassy walls when young. When the mature pycnidium is incubated for 12–24 hours in a moist chamber, the pycnospores are extruded and show the following characteristic development: the wall of the pycnospore turns yellow to reddish brown with hyaline striae; the spore becomes 2-celled by the formation of a septum (Fig. 4). In many cases this transformation into a reddish-brown 2-celled condition takes place while the pycnospores are still enclosed within the pycnidium. This method of spore development is of the same type as occurs in several species of *Botryodiplodia* from the immature *Sphaeropsis*-like forms. The pycnidial stage of the charcoal-rot-inciting fungus, until now classified under *Macrophomina phaseoli*, belongs to the genus *Botryodiplodia*, the immature 1-celled spores being similar to those of *Sphaeropsis*. Detailed examination of pycnidial stages occurring naturally on stems of jute, potato, sun-hemp, and kernels of maize showed that the pycnospores become 2-celled and reddish brown on incubation in a moist chamber, indicating that the fungus under study is a species of *Botryodiplodia*.

Single pycnospores of *Botryodiplodia* described above, were transferred to potato-dextrose agar and incubated at 28°C. The hyphae grew vigorously and produced the characteristic sclerotia. Young hyphae from 48 hour-old cultures, when inoculated into potato tubers by the method already described, produced charcoal rot symptoms within 4 days. It is therefore evident, that the *Botryodiplodia* spores on germination give rise to sclerotium-bearing mycelia. Examination of pycnidia produced in culture also showed 2-celled *Botryodiplodia* spores in association with young 1-celled hyaline spores.

The nomenclature problem of the charcoal-rot-inciting fungus is already in a state of confusion and it is not the object of the present paper to add to it. Ashby (1) and Young (8) have reviewed the nomenclature and pointed out the need for avoiding frequent changes in names of well known pathogens. It must however be pointed out that by preserving a wrong name for purposes of convenience, the essential characteristics of the fungus, which may have a bearing on the life cycle, may be overlooked. For instance, if the conidial stage is a species of *Cercospora*, one will search for a *Mycosphaerella* for the ascigeral stage. Similarly if the pycnidial stage is a *Botryodiplodia*, the perithecial stage may prove to be a species of *Physalospora*.

The sclerotial stage of the fungus first named *Sclerotium bataticola* Taub. was later transferred under *Rhizoctonia* as *R. bataticola* (Taub.) Butler. The pycnidial stage was described by Maublanc (4) as *Macrophoma phaseoli* Maubl. and later as *Macrophomina phaseoli* (Maubl.) Ashby by Ashby (1). The genus *Macrophomina* established by Petrak (5) was reduced to synonymy later by Petrak himself. The hyaline glassy 1-celled spores which become reddish brown and 2-celled at maturity confirm the fact that the fungus is a species of *Botryodiplodia*, the immature stage resembling the species of *Sphaeropsis*. The charcoal-rot-inciting fungus is transferred under *Botryodiplodia* as *B. phaseoli* (Maubl.) Thirumalachar comb. nov.

CENTRAL POTATO RESEARCH INSTITUTE
PATNA, BIHAR
INDIA

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LEAFHOPPER TRANSMISSION OF A DEGENERATION OF GRAPE IN FLORIDA AND ITS RELATION TO PIERCE'S DISEASE [1]

Warren N. Stoner [2]

Attempted grape culture in Florida has a long history dating from the early Spanish settlement in the sixteenth century. The settlers observed, as one may do today, the vigorous growth of the native wild grape, *Vitis simpsonii* Munson, and reasoned that the European grape, *Vitis vinifera* L., should do well. Florida climate and soil seemed suitable and the varieties with which they were familiar were introduced. These early plantings failed, as have all succeeding attempts to grow varieties of *V. vinifera* in the State.

Many varieties of *Vitis labrusca* L., for example Concord, Niagara, Worden, Fredonia and Portland and most of the Texas Post Oak Hybrids such as Extra (Florida Beacon), Carman, Bailey, and Muench have been given extensive field trials in experimental and commercial plantings and all have proved unsatisfactory. Sufficient tests have been made of all types of grapes so that it has been possible to erect groups, using longevity as an index of tolerance to degeneration. Stoner, Stover, and Parris (12) noted this grouping in 1951. To date, none of the accepted commercial varieties have lived long enough to make grapes a reliable phase of Florida's agriculture.

Failure of vines has been progressive throughout the larger vineyards. Terms like degeneration, running out, and exhaustion have been used to designate the condition. These terms are descriptive of individual vine failure, but do not explain the cause.

Several factors have been proposed as likely causes of grape degeneration: improper fertilization, faulty dormancy, insect attack, and others. Parris (8) summarized past investigations of the problem in 1951 and pointed out that none of the fertilization and minor element studies had produced a fertilizer program capable of alleviating grape degeneration. He further stated that soil fumigation in locations where degenerate vines had been pulled failed to protect replants, and that no visible pathogen has been consistently associated with degeneration. Drowning has been eliminated as a cause of degeneration, since the condition kills vines even on well drained slopes. Neither top- nor root-feeding insects have been directly implicated in degeneration. In 1924, Hawkins planted nearly 100 varieties of *V. vinifera* on land known to be free of grape phylloxera, yet all died of degeneration. Moreover, all varieties grafted to phylloxera resistant rootstocks suffer from degeneration. Faulty dormancy, based on the theory that there is insufficient cold for grapes in Florida, has been refuted by observation of isolated individual vines that have survived and produced well. Grapes derived from *V. vinifera* are produced in Nassau; *V. vinifera* varieties are grown extensively in the Mediterranean area and the Orient. These areas have mild climates and are never subjected to low temperatures. Thus, observation and repeated experimentation have shown that none of the proposed causal factors alone or in combination could account for the general widespread occurrence of grape degeneration in Florida.

Parris' (8) description of grape degeneration indicated that symptoms of the condition are similar to those caused by Pierce's disease virus in grape as described by Hewitt et al (5). Field observations and laboratory investigations were made by Stoner, Stover, and Parris (12) during the summer of 1951 on grapes growing on the Whitney farm of the Watermelon and Grape Investigations Laboratory. The notes of the observations and the past history of degeneration coincide with the known facts of Pierce's disease.

Further field plot observations of Stoner and Stover (11) in 1951 and Stoner (10) in 1952 made possible a further comparison of degeneration in Florida with Pierce's disease in California. That comparison is summarized as follows: 1) Past histories of both are almost coincident chronologically. 2) External field symptoms are strikingly similar if not identical. 3) Species of sharpshooters closely related to known vectors of Pierce's disease virus occur in Florida where grape degeneration has long been a problem. 4) Plants proven to be hosts of Pierce's disease virus and its vectors are abundant where degeneration occurs and exhibit symptoms typical of those caused by Pierce's disease virus. 5) Laboratory studies show that tissue aberrations in the internal anatomy of grapes suffering from grape degeneration in Florida are similar to those induced by infection with Pierce's disease virus in the same host in California (1). The overall field ecologies of Pierce's disease and grape degeneration were much too similar to be ascribed to coincidence; hence, in 1951 experiments were started to determine whether or not grape degeneration in Florida is caused by Pierce's disease virus.

MATERIALS AND METHODS.—*Test plants.*—The grape plants used were grown in pots in insect proof greenhouses from healthy cuttings of the Palomino and Zinfandel varieties of *V. vinifera*, except for 1 lot of seedlings noted in the text. Alfalfa plants, variety Cali-

[1] Accepted for publication July 30, 1953.

Florida Agricultural Experiment Station Journal Series, No. 162. The writer wishes to thank N. W. Frazier, J. H. Freitag, M. W. Gardner, Wm. B. Hewitt, B. R. Houston and E. S. Sylvester for their helpful comments and criticisms of the manuscript. The investigations reported have been made in cooperation with the Watermelon and Grape Investigations Laboratory, Leesburg, Florida. The writer is also indebted to Loren H. Stover for much unpublished information of the past history and behavior of Pierce's disease in Florida.

[2] Formerly Assistant Plant Pathologist, University of Florida, Everglades Experiment Station, Belle Glade, Florida.

fornia Common, used in the vector trials were also grown in greenhouses from seed.[3] Control plants equal in number to the test plants in each experiment all remained normal.

Virus sources.—Several vines showing definite symptoms of grape degeneration in the plantings on the experimental farm of the Watermelon and Grape Investigations Laboratory at Whitney, Florida, were marked. These vines were chosen in the early stages of degeneration so that they could be expected to live, under continuous observation, at least through the first year of the experimentation. Material from these indexed vines was used as the virus source for mechanical inoculations and grafting experiments; insects used in the vector trials were fed upon shoots taken from them. The insects used as possible vectors in the field infectivity trials were collected in the vicinity of degenerate vines from Bermuda grass, *Cynodon dactylon* (L.) Pers., a known symptomless carrier of Pierce's disease virus (3, 14).

Mechanical inoculation.—One series of mechanical inoculations was made to grape using the carborundum method of Rawlins and Tompkins (9). Leaves of the test plants were lightly dusted with 600 mesh carborundum powder then gently rubbed with a cotton swab saturated with sap freshly extracted from a composite sample of leaves from the indexed vines used as virus sources.

Grafting technique.—All grafting tests were made by means of chip buds. The top 2 in. of soil were removed from pots in which a rooted cutting of the test grape was growing. A chip was made low in the wood of the rooted cutting and a bud taken from the indexed vines, was inserted. A grafting rubber was then tied to hold the bud in position, and the pot filled with clean sharp sand. The plants were placed in the greenhouse and periodically observed for union of bud and stock.

Vector trials.—Three species of sharpshooters closely related to vectors of Pierce's disease virus listed by Frazier and Freitag (2) were found in grape degeneration areas in Florida. These were *Carneocephala flaviceps* (Riley), *Draeculacephala portola* Ball and *Draeculacephala inscripta* Van Duzee.[4] Only *C. flaviceps*, the yellow-headed sharpshooter, was plentiful and was tested as a possible vector, since it is so closely related to *Carneocephala fulgida* Nott., one of the principal vectors of Pierce's disease virus (5). The insects were collected with a sweeping net from Bermuda grass near Leesburg, caged and transported to Belle Glade, where they were transferred to appropriate test plants.

RESULTS.—*Mechanical inoculation trial.*— Eighteen plants of the variety Emperor were inoculated by the carborundum method as noted in the discussion of materials and methods. A like number of check plants of the same variety was inoculated in the same way using distilled water instead of diseased plant sap. All 36 plants were normal in appearance after being held for observation for 18 months, indicating that no transmission was effected by mechanical inoculation.

Grafting trials.—Two Palomino and 2 Zinfandel grape cuttings were chip grafted from each of 8 different vines showing degeneration symptoms in the field. Sixteen of the 32 grafted plants died, and the grafts failed on 11 of the others. Five successful grafts were obtained from 4 different virus-source vines of the varieties R. W. Munson, Golden Muscat, Ribier, and Extra (Florida Beacon). All 5 plants exhibited typical symptoms of Pierce's disease from 4 to 8 months after grafting. Microscopic examination of stem cross-sections of the 5 grafted plants showing foliage symptoms demonstrated the presence of tyloses and gumming in the xylem elements such as reported by Esau (1) as occurring in grapes infected with Pierce's disease virus. No fungi or bacteria were observed in the sections.

Vector transmission of virus.—Carneocephala flaviceps (Riley), the yellowheaded sharpshooter, the only suspected vector species occurring in abundance in the experimental vineyards at Whitney, Florida, was used in all the vector studies. Since *C. flaviceps* had not been reported as a vector of Pierce's disease virus, it was necessary to test the species. Field collected insects were caged for 24 hours on shoots cut from vines showing symptoms of degeneration in the field. The insects were then transferred to grape test plants and left undisturbed until they died. Table 1 gives the varieties used for virus sources, and the detail of the transfers of 17 tests made in July and August of 1951. The test plants were held in the greenhouse for observation. By June, 1952, 5 showed symptoms typical of those caused by Pierce's disease virus (Fig. 1). Most of the virus source material was in poor condition for feeding and only limited numbers of vectors survived the initial 24 hour period on the source plant pieces. For this reason only small numbers of surviving vectors were available for transfer to certain test plants (Table 1).

Vector field infectivity tests.—Alfalfa plants started from seed in the greenhouse in July 1951 were large enough for vector inoculation by the end of October. Specimens of *C. flaviceps* were collected from Bermuda grass growing in the vicinity of known degenerate grapes. Lots of 15 insects were transferred to each of 6 potted Palomino grape cuttings, 6 Zinfandel grape cuttings, and 19 alfalfa plants in September 1951. The insects were removed at the end of 10 days, and the plants held in the greenhouse for observation. By June, 1952 symptoms resembling those induced by Pierce's disease virus appeared in 2 of the Palomino cuttings and in 4 of the Zinfandel test grapes. Six of the 19 alfalfa plants showed dwarfing symptoms. By November 1952, 10 of the 19 alfalfa

[3] Healthy grape propagation wood, grape seedlings, and alfalfa seed were furnished the author by J. H. Freitag and Wm. B. Hewitt, University of California.

[4] The author is indebted to Professor Dwight M. DeLong, Ohio State University, for determinations of the leafhopper species.

FIG. 1. Representative plants from an experiment demonstrating transmission of Pierce's disease by *Carneocephala flaviceps*. ABOVE: inoculated with a group of 10 insects fed for 24 hours on diseased shoots of the variety Carman; symptoms illustrated developed 11 months after exposure to the viruliferous insects. BELOW: healthy control. Photograph by Grant E. Averill.

plants developed top symptoms of alfalfa dwarf (Fig. 2). The plants were dumped from the pots and the roots were washed and cut for internal examination. All 10 plants showing dwarf top symptoms also had the yellow to brownish discoloration of the root stele associated with dwarf disease of alfalfa. Microscopic examinations were made of the roots and no fungi or bacteria were found.

TABLE 1.—*Results of tests with Carneocephala flaviceps (Riley) as a possible vector of Pierce's disease virus. Insects collected in the field were used in the tests. Microscopic examination of stem cross sections of the plants that developed foliage symptoms disclosed tyloses and gumming in the xylem elements*

Grape Variety Virus Source	Test Plant	No. of Vectors [a]	Days Vectors on test plants [b]	Transmission
Pearl of Csaba	*Vinifera* Seedling	7	22	Negative
Pearl of Csaba	*Vinifera* Seedling	6	24	Negative
Pearl of Csaba	*Vinifera* Seedling	4	17	Negative
Black Spanish	*Vinifera* Seedling	1	3	Negative
Golden Muscat	*Vinifera* Seedling	5	17	Negative
Golden Muscat	*Vinifera* Seedling	5	24	Pierce's disease
Golden Muscat	*Vinifera* Seedling	9	22	Negative
R. W. Munson	*Vinifera* Seedling	5	24	Negative
Carman	Palomino Grape Cutting	10	17	Pierce's disease
Carman	Palomino Grape Cutting	10	13	Negative
Carman	Zinfandel Grape Cutting	10	14	Negative
Carman	Zinfandel Grape Cutting	10	13	Pierce's disease
Florida Beacon	Palomino Grape Cutting	10	10	Pierce's disease
Florida Beacon	Palomino Grape Cutting	10	13	Negative
Florida Beacon	Zinfandel Grape Cutting	10	6	Negative
Florida Beacon	Zinfandel Grape Cutting	12	8	Negative
Golden Muscat	Palomino Grape Cutting	5	13	Pierce's disease

[a] Numbers in this column indicate the number of insects transferred to test plant after 24 hours of feeding on the respective virus source.

[b] Numbers in this column indicate the number of days the last insect of the groups transferred survived.

FIG. 2. Representative alfalfa plants, of the variety California Common, used in testing field-collected insects for ability to transmit Pierce's disease. LEFT: inoculated with a group of 15 *Carneocephala flaviceps* collected from Bermuda grass near degenerate grapes; dwarf disease symptoms illustrated developed 12 months after exposure to the viruliferous insects. RIGHT: healthy control. Photograph by R. J. Allen, Jr. and V. L. Guzman.

Intertransmission studies.—Hewitt et al (6) have shown that the virus causing Pierce's disease of grape also causes dwarf disease of alfalfa. A limited number of intertransmission tests between grape and alfalfa were made with *C. flaviceps*, concurrently with the other vector transmission studies of grape degeneration. This was done so that in the event the sharpshooter proved to be a vector additional information might be available as to the identity of the virus. Field-collected insects were caged for 3 days on shoots taken from the indexed vines showing degeneration symptoms in the field. Lots of 10 insects were then transferred to 1 test grape and 2 alfalfa plants. At the end of 10 days the live insects on the first test plants were transferred to the alternate test plant. Such alternate transfers were continued every 10 days until all insects were dead. The 5 test grapes used in the series developed foliage symptoms similar to those seen in typical cases of Pierce's disease. Three of 5 alfalfa plants in the test developed both top and internal root symptoms similar to those found in alfalfa dwarf. Table 2 lists the order of transmissions in the tests.

DISCUSSION.—Successful transmission obtained in the grafting trails demonstrates that degeneration of grapes in Florida is caused by a virus infection. Transmissions effected with the leafhopper *C. flaviceps* in the vector trials, field infectivity, tests, and intertransmission studies prove this species is a vector of the virus. Failure to transmit the virus mechanically and its retention by the vector in the intertransmission studies indicate that it is a persistent virus as defined by Watson and Roberts (13). Symptoms seen in the field in grape, and those induced experimentally in both grape and alfalfa in grafting trials and in all phases of the vector studies, were identical to those produced in these hosts by Pierce's disease virus.

The foregoing characteristics of the virus causing grape degeneration in Florida conform generally with those of the genus *Morsus* described by Holmes (7), and particularly to the species *suffodiens* of this genus. The author therefore believes that degeneration of grapes in Florida is caused by Pierce's disease virus, *Morsus suffodiens* Holmes.

The data demonstrate that the yellow-headed sharpshooter, *Carneocephala flaviceps* (Riley), transmits Pierce's disease virus, and it is believed that this species is a hitherto unreported vector of the virus. *C. flaviceps* is contained in the subfamily *Tettigellinae* and its addition to the known list of vectors of Pierce's disease virus offers additional evidence that the ability to transmit this virus may be correlated with phylogenetic relationship within this subfamily, as indicated by Frazier and Freitag (2), and Freitag, Frazier, and Flock (4).

SUMMARY

Production of grapes in Florida has been impossible in the past because of a decline in vigor and death of

TABLE 2.—*Detail of intertransmission tests. Test plants developed symptoms 6 to 9 months after insect inoculation. Vectors fed for 3 days on shoots of degenerate grape prior to transfer to first test plant*

Vector Lot No.	No. of Vectors [a]	Days of Plant	Successive Test Plants	Symptoms Developed
1	10	10	Alfalfa	Dwarf
	6	10	Palomino Grape	Pierce's Disease
	4	10	Alfalfa	Dead [b]
	4	4	Palomino Grape	Pierce's Disease
2	10	10	Palomino Grape	Pierce's Disease
	3	10	Alfalfa	Dwarf
	3	10	Palomino Grape	Pierce's Disease
	1	6	Alfalfa	Normal
3	10	10	Alfalfa	Dwarf
	5	8	Palomino Grape	Pierce's Disease

[a] Numbers in this column indicate live insects transferred each time. Thus each number indicates the number of live insects taken from the preceding test plant after the indicated interval of feeding.
[b] This test plant died during the incubation period.

the vines. This condition has been termed degeneration, but the cause was unknown. Recent field observations and laboratory investigations indicated that grape degeneration was possibly due to Pierce's disease virus since no visible pathogen has been consistently associated with the condition, and the field ecologies of both are similar.

Mechanical inoculations with sap from degenerate grapes failed to infect test grapes. The grafting studies reported show that grape degeneration is graft-transmissible and symptoms characteristic of Pierce's disease develop in healthy grapes grafted with buds taken from degenerate vines.

Insect vector trials with the yellow-headed sharpshooter, *Carneocephala flaviceps* (Riley), have proved that healthy grape plants decline and die with symptoms identical with those induced by Pierce's disease virus, after being inoculated with insects previously fed on degenerate vines. Alfalfa test plants develop symptoms of alfalfa dwarf disease after being similarly inoculated.

Field-collected *C. flaviceps* were fed on test grape and alfalfa plants. After a suitable incubation period the plants developed symptoms similar to Pierce's disease and alfalfa dwarf disease.

Intertransmission tests using *C. flaviceps* as the vector and feeding the insects alternately on test grape and alfalfa plants, after an infective feeding on degenerate grape plants, proved that symptoms identical with those of Pierce's disease and alfalfa dwarf can be produced alternately in the respective hosts after such inoculations.

EVERGLADES EXPERIMENT STATION,
UNIVERSITY OF FLORIDA,
BELLE GLADE, FLORIDA

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SUGGESTIONS FOR MORE COMPLETE CONTROL OF DOWNY MILDEW OR BLUE MOLD OF TOBACCO[1]

W. D. Valleau

In any plant disease control program every effort should be made to inform the grower as completely as possible of the nature of the disease, and the ways in which he can help control it by disrupting the normal life cycle of the causal agent. For example, a fruit grower familiar with the life history of the apple scab fungus and mechanically minded might rig up a vacuum cleaner that would collect all dead leaves in the orchard in the fall and thus eliminate carry-over of the scab fungus in his orchard; or the back-yard gardener can control cherry leaf spot effectively by raking and burning leaves in the fall. Similarly, any tobacco grower with proper knowledge of the means of overwintering of the downy mildew or blue mold fungus (*Peronospora tabacina* Adam) can, with comparatively little labor, be certain that his is not a farm on which a blue mold outbreak can start. With joint effort by the majority of tobacco growers in the region it follows that losses from blue mold can be reduced, perhaps to the vanishing point. If such a program is basically sound and practicable, it should be made a part of the present blue mold control program and be vigorously prosecuted. Furthermore, if it is only necessary to make this effort in a comparatively small portion of the tobacco growing area, in order to relieve the remaining hundreds of thousands of growers in the United States and Canada of yearly worries and losses, it would seem highly desirable that a whole-hearted effort be made to do the job.

It is the intention, in this paper, to present evidence that if spore showers from farther south can be prevented, the blue mold fungus is incapable of perpetuating itself year after year in areas where above-ground parts of tobacco plants are regularly killed by frost thus limiting survival of the fungus to oospores[2] in beds used a second time. Suggestions will also be made for the elimination of spore showers from the South, and the eventual elimination of the fungus.

Blue mold was found in the Florida-Georgia shade tobacco area in 1921, when it spread to practically all tobacco beds in the area. The disease was investigated by Smith and McKenney, who proposed an aggressive campaign against the fungus (11). Shade tobacco growing is a highly specialized industry so it is probable that Smith's recommendations were followed, at least insofar as cleaning up affected beds and sterilizing the beds for the following year were concerned. Whether because of this or for some other reason, the fungus was completely eradicated from the area, for it did not appear again until 1931. In 1931 spores were again blown in, presumably from Texas where the fungus is abundant on the wild *Nicotiana repanda* (12). This time the disease appeared in Georgia in flue-cured tobacco north of the shade tobacco area. By 1931 the flue-cured industry had been introduced into southern Georgia, which made conditions for the survival of the fungus considerably different from those in 1921. Since that time the disease has been a menace to nearly the entire tobacco industry in the eastern part of the United States and Canada, not only causing large losses some years but causing the expenditure of enormous amounts of labor and cash for chemicals in an attempt to control it.

The only tobacco industry that has not suffered during the past 21 years is the Perique of Louisiana. The disease appeared in Perique tobacco beds in 1931 (4), but did not reappear in 1932 (1) and has not been seen since. The reason for its disappearance, as determined by the writer, is that the growers are all market gardeners who destroy tobacco plants in the beds following setting and use a different bed the following year. It is impossible, under these conditions, for the fungus to persist as a pathogen of cultivated tobacco.

The tobacco growing regions of North America can be divided into 3 distinct areas so far as blue mold is concerned:

Area 1. Georgia-Florida, where the fungus lives into the winter on or in the ground as oospores, and may live through the winter on volunteer or surviving tobacco plants in a bed used the previous spring, or on suckers from the previous tobacco crop. Blue mold on these plants initiates early outbreaks in plant beds when plants are small.

Area 2. The states to the northeast of Georgia, including the Carolinas, the Virginias, Maryland, and Pennsylvania. It is an area that is subject to spore showers from farther south as well as carry-over by oospores in beds used a second season. In this area it is difficult to determine the relative parts that spore showers from the South and oospore carry-over in second-year beds play in a general outbreak and in the persistence of the fungus in the area.

Area 3. The Tennessee, Kentucky, Ohio, Indiana, and Ontario, Canada area. This area is out of the direct line of spore showers from the South with the possible exception of East Tennessee, but the fungus overwinters in some beds used the previous season. So far, 2 heavy spore showers have occurred in Area 3 in 22 years—one in 1937 and in 1945. Following both spore showers there were general outbreaks for

[1] Accepted for publication July 27, 1953.
The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the Director.

[2] It is assumed, in this paper, that the means of overwintering, in beds used a second time in areas where no above-ground parts of tobacco plants survive the winter, is by oospores, as no other means is known and oospores are sometimes produced in abundance.

2 years, followed by a gradual decline until the fungus practically disappeared. Thus it has been possible, in Area 3, to evaluate the relative importance of spore showers and carry-over in plant beds in perpetuating the fungus from year to year. Without spore showers from the South, the fungus cannot perpetuate itself in this area for an extended period, and particularly so if growers use new bed sites.

What happened in the Tennessee-Kentucky area (Area 3) following the spore showers of 1937 and 1945 will illustrate what can be expected in the Carolinas and north (Area 2) if spore showers from the South can be eliminated. Whereas blue mold became established in Georgia in 1931, it was not until 1933 that any blue mold appeared in Tennessee, when it was found in 22 east and middle Tennessee counties (10). In the 1934 survey it was not found (2). In 1936, a very dry season, a few infections were found in shaded beds in southern Kentucky a couple of weeks after setting was completed. In 1937 there was a general outbreak in Tennessee and Kentucky, evidently the result of spores blown in from the Southeast, as infections occurred simultaneously over a wide area. In 1938 the disease was general in Kentucky, a situation very similar to what occurs every year in Area 2, the Carolinas. In 1939 it was reported from only 15 Kentucky counties, with carry-over in at least 19 beds. There was very little spread that year and consequently little carry-over to 1940. By 1944 the fungus had practically diseappeared from Tennessee, Kentucky, Ohio, and Indiana, as only 2 plant bed sites in Tennessee were found to be infected during an extensive survey in Tennessee and Kentucky, made by Dr. Hyre of the Plant Disease Survey (9). The disease had disappeared several years earlier from Ontario, Canada according to information furnished the writer by Dr. Koch in 1944.

In 1945 another blow-in of spores into Area 3 occurred, presumably from Area 1 or 2.[3] This resulted in a general outbreak in Tennessee, Kentucky, Ohio, Indiana, and Ontario. In 1946, county agents were asked to be on the lookout for the first outbreaks in Kentucky and to determine whether they were in new beds or second-year beds. The evidence was quite clear that the 1946 outbreak was from carry-over in second-year beds. During the next 2 years there was clear-cut evidence of carry-over in second-year beds, but the fungus was rapidly disappearing. In 1952 blue mold was reported from only a few beds in seven counties in Kentucky at setting time, too late to cause any injury and the same was true in 1953. It had been evident since 1948 that the fungus could not maintain itself. It also disappeared from Indiana, Ohio, and Ontario. These results, covering a period of 16 years, seem to demonstrate conclusively that the blue mold fungus is not capable of maintaining itself over an area of several states, provided it is dependent entirely on oospore carry-over in plant beds for its survival. Dixon et al (3) demonstrated in North Carolina in 1934 that only about 35 per cent of beds used a second year showed evidence of infection from carry-over. Of course carry-over could be greatly reduced in that area by the use of new bed sites or the use of some means of partial sterilization of beds, such as methyl bromide, that might destroy the overwintering spores. In Kentucky, growers who use the same bed site year after year, sow soybeans after setting is completed, plow the soybeans under in the fall, and steam, burn, or treat with methyl bromide, have had no trouble whatever from carry-over.

Dixon et al (3) also demonstrated that in 1934 infection from carry-over appeared in old beds several days before infection appeared in new beds or before there was evidence of a spore shower, and so it might be concluded that spore showers were of little importance in the Carolina area. However, it must be kept in mind that in 1931, when blue mold first appeared in Georgia as late as early April, it advanced north at the rate of about 75 miles per week, finally reaching Maryland, causing a fairly general outbreak as it advanced. If an outbreak as late as early April in Georgia could have such an effect, it is probable that the outbreaks that start in Georgia in January or February play a much greater role in the severity of the disease in the Carolinas and north. Sufficient spores fall on beds to guarantee that all new beds as well as old ones are infected each year, thus increasing carry-over.

What are the prospects of eliminating spore showers from the Georgia-Florida area? As has been mentioned, blue mold is carried through the winter in the Georgia-Florida area in 2 ways: on living plants, either suckers from the previous crop or on old or volunteer plants in last season's bed, and as oospores in beds used a second season. If the fungus lives through the winter on living plant parts, it means that conidia are being distributed in time to cause infection on very young plants in new beds. If the outbreak is limited to oospore carry-over, an outbreak usually does not get well under way until about setting time.

Gaines (5,6,7,8) on several occasions, has reported finding blue mold on plants in Georgia in old beds when plants in new beds were large enough to become infected. Dixon et al (3) reported that suckers affected with downy mildew had been found in midwinter in southern Georgia. There have been few reports of the fungus overwintering on live plants, suggesting that blue mold rarely overwinters in this fashion but that in the few places each year where it does it must play an important role in initiating early outbreaks.

It should not be difficult to eliminate overwintering suckers in flue-cured tobacco fields because the crop is harvested early in the summer and there is a long hot period when the surviving plants could be killed. It is no problem for the individual farmer to destroy

[3] On a visit to Texas made by the writer it was determined that blow-in could not have occurred from Texas, as by the time tobacco plants in Area 3 are established in the plant bed, temperatures in Texas are high and the fungus has stopped sporulating on *N. repanda* completely.

the remaining plants in his bed when he is through setting his crop. It would pay him from the standpoint of insect control alone, if for no other reason. If all living tobacco plants are destroyed in the Georgia area as far north as suckers and volunteer plants normally survive, early outbreaks of blue mold should be eliminated immediately; and even though compliance was not 100 per cent, the severity of an outbreak would be reduced with the elimination of each bed that would have carried the fungus over winter. Then, if growers in this area used new bed sites there is no other known source from which an outbreak could occur.[4] As to costs, it will be far less costly to disk a tobacco bed after the crop is set and use a new site the next year than to dust or spray beds with ferbam or some other chemical, particularly as one program would lead to the diminution and perhaps elimination of the fungus, while the other could only look forward to dusting or spraying ad infinitum.

The means of instructing farmers on this phase of blue mold control quickly and effectively are at hand. In each state there is a well organized extension service capable of doing a thorough job at little if any extra cost. All that is necessary is that competent authorities in each state involved determine what would be the most effective procedure in that state, instruct extension specialists and county agents, and then put on an aggressive educational campaign. Tobacco allotments are valuable, the acreage that a grower can raise is limited, and so growers can be expected to do whatever will, in the long run, cut down costs and increase profits on the limited tobacco acreage available, as this is often his greatest source of cash income.

The present dusting or spraying program should be continued as long as it is necessary, as it is not only helpful in providing plants for the current crop but should help reduce the magnitude of spore showers and reduce the number of oospores carried from 1 season to the next; but it should be supplemented by recommendations to destroy beds after the crop is set, to destroy overwintering plants, and to use new bed sites.

If the program is put on, it should not be expected that the fungus will disappear immediately, as it is not likely that all growers will comply; but growers particularly in Georgia, should be constantly reminded that they can prevent carry-over on their own farms, and that when enough do the blue mold disease will cease to be a problem. If this program is effectively carried out in Georgia, it can be anticipated that it will be increasingly difficult for the fungus to survive farther north by oospores alone, and will tend eventually to disappear as it has in the Tennessee-Kentucky area.

[4] According to information furnished by R. R. Kincaid, J. K. Small, in his Manual of Southeastern Flora, 1933, lists *N. rustica*, *N. longiflora*, *N. repanda*, and *N. glauca* as being present in Florida or eastern United States. It would be expected, if they harbor the fungus, that tobacco when first introduced into Florida would have been affected; that the fungus would not have disappeared from either Georgia or Florida after 1921, or from Louisiana after 1931. So it seems probable that wild species play no part in survival east of Texas.

SUMMARY

There are 2 known ways in which the blue mold fungus survives from year to year; one is on live plants that survive the winter in Georgia, and the other is by means of oospores in beds used a second year. Elimination of these 2 means of overwintering in Georgia should immediately eliminate the fungus in that area. In the Tennessee-Kentucky area, which is out of the direct line of spore movement north from Georgia, the fungus is dependent almost entirely on oospores for survival and is not able to maintain itself year after year. The Carolina-Virginia area is subject to spore showers from the South as well as infection from overwintering oospores; consequently the fungus persists year after year. Presumably the elimination of spore showers from the South in the Carolinas would result in the gradual disappearance of the fungus there just as in the Tennessee-Kentucky area. The proposal to use new beds and destroy overwintering tobacco plants is practical in view of the well organized extension services in the states concerned.

Department of Agronomy
University of Kentucky
Lexington, Kentucky

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THE WATER ECONOMY OF FUSARIUM WILTED TOMATO PLANTS[1]

A. E. Dimond and Paul E. Waggoner

Recent studies on Fusarium wilt of tomato, caused by *Fusarium oxysporum* f. *lycopersici*, have presented evidence of a water shortage in the plant as a cause of wilting.[2] When Ludwig (6) and Scheffer and Walker (8) applied equal pressure to diseased and healthy stems and petioles, less water moved through the infected vascular system. They reasoned that the water shortage caused by an impediment to water movement was the cause of wilting.

In contrast with the hypothesis that water shortage arises from impediments to flow is the one advanced by Gaumann (3) which ascribes wilting to the formation of lycomarasmin by the pathogen. Lycomarasmin is said to be translocated to leaves where it destroys the differential permeability of parenchyma cells, thus rendering them impossible as an osmotic mechanism.

The present paper examines certain consequences of the hypothesis that water shortage and wilting arise from impediments to normal flow of water in stems and petioles. If this hypothesis is correct, the spongy mesophyll cells in the wilting leaf should still be capable of functioning as an osmotic system. Further, if the decrease in water supply is prolonged, leaves on an infected plant should present the same transpirational picture as those on a drought-hardened plant. Finally, it should be possible to confirm by another method the observation that flow of water through xylem elements is reduced in the diseased stem. These pieces of evidence are the basis of the present study.

THE OSMOTIC PROPERTIES OF LEAF PARENCHYMA IN DISEASED PLANTS.—If, in Fusarium wilt, impediments to water movement in vascular tissue cause a reduction in water flow, then wilting in infected plants should be comparable to that caused by large molecules in the vascular system (4). For example, the petioles and leaflets of a cutting placed in a ¼ per cent pectin solution become flaccid. If a wilted leaf is excised above the point of plugging and the cut end is placed in water, the leaf tissue becomes turgid in a few hours. Thus pectin does not affect differential permeability of leaf cells. This is the manner in which wilted leaves should behave if the hypothesis is correct.

That leaf cells have not lost their ability to function as an osmotic system has been repeatedly demonstrated in the present studies. Flaccid green leaves are removed from a *Fusarium* infected tomato plant. If vascular bundles in petioles are discolored, the leaves will not recover their turgor when petioles are placed in water, but the leaf blade tissue will slowly recover its turgor if it is immersed in water. If, however, the discolored tissue is first removed and the remaining petiolar stub is placed in water, the wilted leaf will recover its turgor in a few hours. Evidently it is necessary only to remove the dysfunctional tissue in order to observe the unimpaired osmotic properties of cells in wilted leaves from diseased plants. These facts must be remembered when one evaluates experiments on Fusarium wilt in which the inability of leaves to regain turgor is cited as evidence for vivotoxins which cause cells to lose their differential permeability (3).

TRANSPIRATIONAL LOSSES FROM LEAVES ON HEALTHY AND DISEASED PLANTS.—An attempt was made in this study to estimate the transpiration rate of the leaf on the intact plant. Leaves were removed from diseased or healthy plants by cutting the petiole just below the lowest pair of leaflets. At this instant an interval timer was started. As rapidly as possible the leaf was hung from the beam of an analytical balance. The balance was then set to a weight very slightly less than that of the leaf. When the pointer on the balance passed the equilibrium position, the time and weight of the leaf were recorded to the nearest second and 0.1 mg. Then the balance was reset to a weight slightly less than that of the leaf and the process was repeated. This operation was continued, to obtain as many readings as possible within a 5-minute period.

Moving averages of each 2 successive observations were computed and the loss in weight of each leaf was calculated in mg./sec. for each time. Then for a given leaf the rate of loss in weight was plotted against time, and the resulting curve showed the relationship between transpiration rate and time. This curve was then extrapolated to zero time, the time when the leaf was detached from the plant. This value was used as an estimate of the rate of transpiration of the leaf when attached to the plant.

In a preliminary experiment the shape of the transpiration rate curves with time was compared under 2 contrasting conditions. In 1 case, tomato stems were cut from the roots and immediately transferred to water for 1 minute. Then leaves were removed from the stem individually and weighed. In the other case, leaves were removed directly from the intact plant and weighed as described above. The transpirational pattern of leaves under these 2 conditions differed considerably (Fig. 1). When leaves were removed from cuttings supplied with water, the transpiration rate was higher initially than it was from leaves removed from intact plants; moreover the transpiration rate decreased linearly with time (Fig. 1, curve 1). When leaves were removed from intact plants, the transpiration rate was low and tended to increase slightly with time (curve 2). The difference in transpiration rate of leaves on the intact plant versus that on cuttings sup-

[1] Accepted for publication July 31, 1953.
Research conducted under Contract No. AT-(30-1)-580 between the Atomic Energy Commission and The Connecticut Agricultural Experiment Station.

[2] In this paper "wilting" means simple loss of turgor.

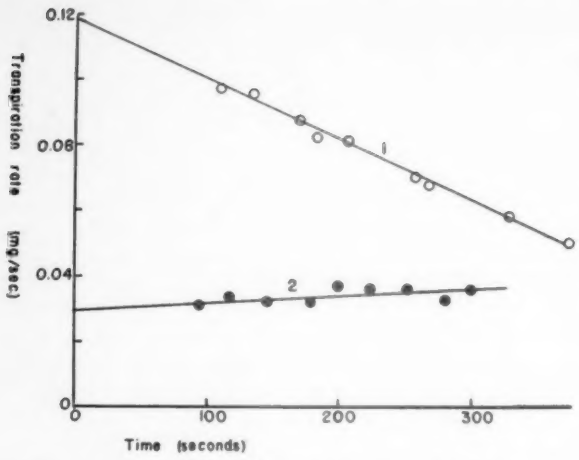

FIG. 1. The change in transpiration rate of tomato leaves with time after removal from the stem. CURVE 1: Leaf removed from a cutting in water for 1 minute after being removed from the plant. CURVE 2: Leaf removed directly from the intact plant.

plied with water reflects the tension on water columns in the intact plant with resultant lower availability of water to the leaf. Obviously, estimates of transpiration rate of leaves removed from cuttings supplied with water must be adjusted for such differences if results are to be applied to the intact plant.

Both methods were used in the present study. In comparisons of the transpiration rates of leaves on healthy and diseased plants, leaves were removed from intact plants and weighed. In estimates of the velocity of the transpiration stream in the intact plant, the transpiration rate of leaves removed from cuttings in water was compared with that of leaves removed directly from plants of the same age and otherwise treated identically.

Leaf areas were then measured by making a contact exposure of the leaf on photographic film, developing it, and after drying the film, cutting out the outline of the leaf. From the weight of the leaf outline on film and reference to a calibration curve in which the weight of known areas of film had been determined, the area of the leaf was obtained. With this information, transpiration rates in mg./cm^2./sec. were obtained.

Half of a lot of tomato plants at the 4-leaf stage were inoculated with *F. oxysporum* f. *lycopersici*. All plants were then transplanted and were supplied with abundant water in an automatically watered greenhouse bench. Two weeks later the leaves of each plant were scored for severity of wilting symptoms and a grade was computed for each plant as a whole, the grades ranging from 0.00 for a healthy plant to 4.00 for one completely killed by Fusarium wilt (2). In the pair of plants to be discussed here (Table 1), the healthy plant had a leaf grade of 0.00 and the inoculated plant a grade of 1.20. Leaves were numbered according to height on the plant, the cotyledons being 0, the first true leaf being 1, etc.

A given leaf on the healthy plant was removed and its transpiration rate determined. Then the transpiration rate on the corresponding leaf of the diseased plant was measured. Within this framework, leaves were removed in random order from a plant. The results of an experiment will illustrate the differences which were obtained in the transpiration rates (Table 1).

Diseased plants transpired at a lower rate than did healthy plants. The diseased plant had 5 leaves which showed no wilting symptoms. These leaves were transpiring more rapidly than the symptomatic leaves were (Table 1). To determine the effect of Fusarium infection in the stem on turgid leaves, the transpiration rate of the 5 turgid leaves on the diseased plant is compared with that of the corresponding leaves on the healthy (succulent) plant. The average transpiration rate of these leaves on the disease plant was 0.65 $\times 10^{-3}$ mg./cm^2./sec. and of the corresponding leaves on the healthy plant was 2.04 $\times 10^{-3}$ mg./cm^2./sec. Thus turgid leaves on the diseased plant transpired 1/3 as rapidly as leaves on a healthy (succulent) plant. Evidently as water movement through the stems

TABLE 1.—*Transpiration rates of leaves on diseased and healthy plants*

	Healthy plant[a]		Diseased plant		
Leaf position	Transpiration mg./sec.	Area cm^2.	Transpiration mg./sec.	Area cm^2.	Leaf symptoms on diseased plant
10	4.8 $\times 10^{-3}$	3.0	3.1 $\times 10^{-3}$	1.1	None
9	10.1	8.1	3.4	4.6	None
8	93.0	22.0	23.7	17.3	None
7	140.0	35.4	13.0	33.8	Moderate wilt
6	82.0	47.2	26.5	52.2	None
5	82.0	52.9	32.0	60.5	None
4	132.0	43.3	9.2	34.4	Severe wilt
3	28.0	40.8	13.0	44.8	Moderate wilt
2	40.0	22.4	10.5	32.4	Severe wilt
1[b]	14.0	8.8	3.4	9.4	Yellow, turgid
A Sum: all leaves	625.9	283.9	137.8	290.5	
B[c] Sum: leaves 5-6, 8-10	271.9	133.2	88.7	135.7	
B/A	0.43	0.47	0.64	0.47	

[a] All leaves green and turgid.
[b] Lowest true leaf.
[c] Sum of turgid leaves on diseased plant or corresponding leaves on healthy plant.

and petioles decreases (6, 8), the transpiration rate of a turgid leaf decreases gradually in the diseased plant.

The difference in transpiration rate of turgid leaves from diseased and healthy plants was obvious at the time the measurements were made. Leaf 5 from the healthy plant became flaccid 5 minutes after it was removed. In contrast, the corresponding leaf from the diseased plant, although turgid when removed from the plant, began to lose its turgor perceptibly only after ½ hour. For this reason the stomates were examined microscopically on leaves still on plants. Leaf 6 was also turgid, both on the healthy and diseased plants. The stomates on the leaf from the healthy plant were open whereas they were closed on the corresponding leaf from the diseased plant. The leaf from the healthy plant again wilted rapidly whereas the leaf from the diseased plant remained turgid for ½ hour when cut from the plant.

These differences suggested that the decreased flow of water through stem and petioles in diseased plants (6,8) would result in a gradually increasing water shortage in leaves. If the water shortage occurs gradually, the leaf will become drought-hardened. As Rosa (7) and Loomis (5) have shown, such tomato leaves transpire more slowly than do succulent ones. The decrease in transpiration from a drought-hardened leaf should be similar in magnitude to that of a leaf on a diseased plant under comparable conditions. This relationship was explored experimentally.

At the same time that studies on the transpiration rate of leaves from healthy and diseased plants were carried out, transpiration studies were conducted on tomato leaves from healthy succulent and drought-hardened plants. These plants were the same age and size as those used in the comparison of diseased and healthy plants, and drought-hardened plants were produced by withholding water from them for 3 weeks, just sufficient being supplied to prevent wilting. When the transpiration rate was determined on comparable leaves removed from intact plants, the transpiration rate of succulent leaves was 4.75×10^{-3} mg./cm^2./sec. while that of drought-hardened leaves was 0.75×10^{-3} mg./cm^2./sec. The drought-hardened leaves were transpiring 1/6 as rapidly as succulent leaves. Moreover drought-hardened leaves remained turgid for as long as 30 minutes after being removed from the plant, just as diseased leaves did, whereas succulent leaves lost their turgor within 5 minutes of being detached.

To determine whether the difference in transpiration rate between succulent and hardened leaves also prevailed when leaves were supplied with abundant water, the transpiration rate was measured when cuttings were first removed to water and the leaves subsequently detached and weighed. Leaves in the same position on each group of plants were used in making this comparison. Under these conditions the transpiration rate of succulent leaves was 9.17×10^{-3} mg./cm^2./sec. and that of hardened leaves was 1.29×10^{-3} mg./cm^2./sec. Evidently the difference in transpiration rate continued, even though the leaves were supplied with abundant water.

Therefore, the decreased transpiration of turgid leaves on infected plants and their ability to remain turgid for ½ hour after being cut from the plant is consistent with the hypothesis that wilting results from impediments to water flow in Fusarium wilt (6,8). The leaves suffer from a drought caused by decreased water flow through the stem, become drought-hardened, and at least a part of the decreased transpiration observed on leaves from diseased plants is ascribable to this hardening.

VELOCITY OF THE TRANSPIRATION STREAM IN HEALTHY AND DISEASED PLANTS.—An estimate of the velocity of water movement through a small tomato stem can be obtained from the use of dilute dye solutions. A healthy tomato plant 20 cm. high was cut at the base of the stem, the cut end quickly placed under water and cut again. To permit tension in the conducting elements to be released, the cutting was allowed to stand for 1 minute under water. It was then transferred to a 1 per cent Eosin B solution for 75 seconds, following which it was rinsed in water and the stem rapidly dissected. Microscopic examination of free hand sections showed the dye to have penetrated all vascular elements excepting only the petiole bundles of the lowest true leaf and the 2 most lateral petiole bundles of the youngest leaf. This suggested at once a rapidly moving transpiration stream in which small as well as large conductive elements of the xylem were functional. The rate of movement was at least 0.27 cm./sec.

To refine this method, radiophosphate (P^{32}) was employed instead of dye and the presence of radiophosphate was detected by a Geiger counter and scaler. The P^{32} was assumed for 2 reasons to move at the rate of the transpiration stream. First, it gave rates of the same order as the dye method. Second, uptake took place over a very short period. The gradient of P^{32} concentration in the stem was from highest at the base of the cutting to lowest at the top. Hence, movement, not accumulation, was being measured. The bulk of P^{32} movement is known to be through the xylem (1). The length of the stem of the test plant was first measured from the cotyledonary node to each of the nodes above. This plant was then cut off just below the cotyledonary node and, after the base of the stem had been in water for 1 minute, the base of the cutting was immersed up to the cotyledonary node for a stated time in a dilute solution of radiophosphate (0.13 μc./ml.). Following exposure, the cutting was rapidly dissected from the top down, cutting the stem just above each node and cutting the base of the stem at the cotyledonary node which was always just at the point of immersion of radiophosphate. Then leaf tissue including petiole ends was rapidly cut off just below the most basal pair of leaflets. Finally the petiole stubs were cut from the stems. Nodal tissue was then cut off each stem segment and discarded.

In these experiments the absorption by plant tissue

TABLE 2. *The rate of movement of radiophosphate through vascular tissue of a healthy tomato plant*

Petiole No.	Distance from cotyledonary node		Counts per minute in sample	
	Stem	Stem + petiole	Stem	Petiole
7[a]	15.8 cm.	17.6 cm.	2 ± 5[b]	0 ± 5[b]
6	14.7	18.1	2 ± 5	9 ± 6
5	12.7	16.6	12 ± 6	9 ± 6
4	10.4	14.4	16 ± 6	17 ± 6
3	7.2	11.2	36 ± 7	8 ± 6
2	4.2	8.2	38 ± 7	6 ± 5
1	2.0	5.5	61 ± 8	3 ± 5[c]

[a] Petioles are numbered in order of their age and height on the plant.
[b] 5 per cent counting error.
[c] The conductive capacity of the oldest petioles on a plant is very much less than for petioles midway up the stem. The diameter of all functional conductive elements in petioles at various heights in a plant bearing 9 leaves was measured microscopically. The total cross section of these elements was then determined for each petiole. In ascending order on the plant, these petioles had conductive cross sections in units of 10^{-5} cm.^{2} as follows: 0.9, 6.6, 12.8, 29.2, 27.2, 40.8, undetermined, 21.4, and 6.5. A low radiophosphate content of the oldest petioles would therefore be expected and was observed. The method of dissecting the plant in preparing samples and the short exposure to radiophosphate (30 seconds) rule out the possibility that phosphate accumulation by young leaves accounts for the observed distribution.

of the medium energy beta rays from radiophosphate was made constant among samples by the method of preparing them for counting. The usual correction for self-absorption is based on the assumption that radioactivity is evenly dispersed in a sample. In the present case this is not true because the radiophosphate is concentrated in vascular bundles which are peripherally distributed about the stem and petiole. For this reason the petiole and stem samples were cut longitudinally and the cut surface placed downwards in 2.5 cm. counting dishes. This procedure resulted in the vascular strands being oriented toward the counting tube; accordingly the radiation had a constant path to travel through plant tissues to arrive at the counting tube.

In a population of uniform tomato plants, some were inoculated and others were not. All were transplanted at this time and grown with abundant water supply in an automatically watered greenhouse bench. Two weeks afterwards 3 plants were graded for severity of foliar symptoms (2). Grades for the 3 plants were 0.00 for a noninoculated plant, 1.25 for a moderately wilted plant, and 2.67 for a severely wilted plant.

The period of exposure to radiophosphate was 30 seconds for the healthy plant and 8 minutes for the diseased ones, preliminary experiments having indicated that in 8 minutes radiophosphate had become distributed through the entire vascular system of a healthy plant but not in a diseased one. After exposure plants were dissected, and samples prepared and counted. Counts were then examined statistically and only counts differing significantly from background counting rate were employed in subsequent computations. To illustrate the method by which flow rates in tomato stems were computed, data obtained from a healthy plant are presented (Table 2).

Conservatively figured, the transpiration stream in the healthy plant had moved radiophosphate as far as the fifth petiole above the cotyledonary node a distance of at least 12.7 cm. and at most 16.6 cm. in a 30-second period of uptake. Accordingly the rate of movement through the stem was, at most, 16.6 cm./30-second or 0.55 cm./second. In similar fashion the rate of movement of material through the stem of a moderately diseased plant was 0.02 cm./second and in a severely diseased plant was 0.01 cm./second. Thus the rate of water movement through a moderately diseased stem was 4 per cent and through a severely diseased stem was 2 per cent of that in a healthy one. These observations are consistent with what has previously been reported on flow in infected xylem (6,8).

In the above experiments tomato cuttings were used in order to make definite the moment when radiophosphate passed a known point in the vascular tissue, a matter which would not be possible if intact plants had been used. The relative rate of movement in healthy and diseased tomato stems is obviously not altered by using cuttings instead of intact plants, but the absolute magnitude of the rate of movement of water in stems is found to be lower when intact plants are used instead of cuttings. The increase in apparent rate of water movement in the stem resulting from employing cuttings rather than intact plants can be determined from studies on the transpiration rate. This is so because in the turgid leaf the rate of water movement through xylem elements must equal the rate of water loss by transpiration. Therefore, studies on transpiration rate in cuttings as compared with intact plants provide a direct estimate of the change in rate of water movement through stems when ends of vascular strands are in contact with water as compared with the situation in the intact plant.

Two healthy tomato plants were selected of the same size used in the other experiments described here. One stem was severed at the ground line and immediately cut again at the cotyledonary node under water. The other was left intact. In both cases leaves were removed and the transpiration rate was determined. The average transpiration rate for all leaves was 2.7 times as great for a cutting as for the intact plant (in units of mg./cm^2./sec.). In another experiment the transpiration rate of detached leaves removed to water was 1.7 times that of leaves removed

directly from the plant.

The average of these measurements, 2.2 times the transpiration rate of leaves directly removed from the plant, can be used to correct the estimate obtained with cuttings of flow rate in the stem. The actual velocity of water in an intact healthy stem was 0.55/2.2 or 0·25 cm./sec. The corresponding rate in an intact, moderately diseased plant was 0.0091 cm./sec. and in a severely diseased plant was 0.0045 cm./sec. These estimates of the flow rates in intact healthy and diseased plants confirm previous findings (6,8) that there is an impediment to water movement in infected tomato stems and petioles.

DISCUSSION AND SUMMARY

The cause of wilting in Fusarium wilt of tomatoes has been ascribed to a number of factors. Recent workers (6,8) have observed a decreased flow of water in stems and petioles of diseased plants as compared with that in healthy ones. In these experiments a constant pressure difference was applied to the 2 ends of the cuttings. In the present study the velocity of the transpiration stream was measured by means of radiophosphate movement in diseased and healthy cuttings.

The rate of radiophosphate movement in diseased stems was found to be 0.0091 cm./sec. when the stem was moderately diseased and was 0.0045 cm./sec. when the disease was severe. In healthy stems the rate of solute movement through xylem elements was 0.25 cm./sec. under these conditions. Thus the rate of movement of solutes through xylem in diseased stems is from 2 to 4 per cent of that in healthy stems. The findings of Ludwig (6) and of Scheffer and Walker (8) are thus confirmed.

Waggoner and Dimond (9) have observed small decreases in P^{32} content of the upper parts of diseased relative to healthy tomatoes. However, they used extended periods of exposure to P^{32} (24 to 76 hours). After these extended exposures, the highest concentrations of P^{32} were in the youngest, upper parts of the plant. That is, accumulation rather than uptake was the dominating factor in determining distribution. Apparently the difference between healthy and diseased plants was smaller than described in the present paper because *Fusarium* decreases uptake more than accumulation.

In Fusarium wilted tomato plants there is evidently an impediment to movement of the transpiration stream. Two consequences of this hypothesis are that 1) Fusarium wilted tomato leaves should recover their turgor if the impediment to water movement is removed from conductive elements of the xylem and 2) there should be a gradually increasing water shortage in turgid leaves of the infected plants which leads to drought-hardening and thus affects the transpiration rate of leaves.

A wilting leaf from an infected plant regains its turgor if discolored vascular tissue in the petiole is first removed and the basal end of the remaining petiole tissue is then placed in water. Evidently the leaf cells of wilting leaves retain their ability to function as an osmotic system, and translocatable vivotoxins which destroy permeability of leaf cells are not involved as a cause of wilting.

If an impediment to water flow arises as infection in the stem progresses, leaves will be subjected to a gradually increasing water shortage. These are conditions under which drought-hardening should occur. Turgid leaves on infected stems were found to transpire at ⅓ the rate of succulent tomato leaves on healthy stems. Drought-hardened healthy leaves were found to transpire at 1/6 the rate of succulent healthy leaves. The stomates of turgid leaves on diseased plants remained closed when stomates of healthy leaves were fully opened. As a result of these factors the turgid leaves of both diseased and drought-hardened plants remained turgid for a longer period after being cut from the plant than leaves of normal succulent plants.

Thus the hypothesis that a water shortage in leaves of *Fusarium* infected tomato plants arises from impediments to water flow has been examined from 3 points of view and nothing inconsistent with this hypothesis has been found.

Department of Plant Pathology and Botany
The Connecticut Agricultural Experiment Station
New Haven, Connecticut

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EPIDEMIOLOGY OF FLAX RUST IN THE NORTH CENTRAL STATES [1]

H. H. Flor [2]

Rust, *Melampsora lini* (Pers) Lév., has been the most destructive disease of flax in the North Central States since the early 1920's when wilt resistant varieties were developed. It is widely prevalent and locally destructive every year and in 1928, 1941, 1942, 1950, and 1951 caused losses of epidemic proportions.

The epidemiology of cereal rust has received extensive study (1, 11, 12). Flax rust differs from the rusts of cereals in a number of important respects that affect its epidemiology.

Flax rust is eu-autoecious, and it is not possible to prevent hybridization of races by destroying an alternate host, as can be done with the cereal rusts. Because the urediospores do not overwinter in the North Central States (10) hybridization probably is concomitant with the initiation of each year's infection. Rust seldom is important in the limited area of Texas where flax is grown as a winter crop. Great distances separate the winter and summer flax-growing areas and it is unlikely that wind-borne spores from the south are a factor in the epidemiology of flax rust. Even in the North Central States, flax fields are dispersed and early infections are of local origin. By good cultural practices and proper sanitary measures some farmers continue to grow rust-susceptible varieties successfully.

This paper reports the results of physiologic race surveys from 1931, when physiologic specialization in the flax rust fungus was first demonstrated (3), to 1951, inclusive. Consideration is given to changes in the pathogenicity of races during this period, to the discovery of races carrying new genes for virulence, to the effect of the interaction of races and flax varieties on the survival of each, and to the spread of new races.

METHODS.—New developments have been incorporated into the scheme of race identification as they have been discovered, in order to facilitate the production of rust-resistant varieties. The differential varieties were selected by testing the reaction of the varieties grown in the 1931 Flax Classification Nursery of the United States Department of Agriculture to field collections of rust (3, 4). Studies on the interaction of genes conditioning rust reaction in flax (6) with those conditioning pathogenicity in the rust fungus (5) showed the advantages of using varieties with single rust-conditioning genes. Because resistance to rust has invariably been dominant, a variety with more than 1 rust-conditioning gene cannot differentiate races attacking none of its genes for rust reaction from races attacking any combination of these genes except the combination including all these genes. Morye, a variety with 4 rust-conditioning genes, is resistant to all races attacking any combination of 0, 1, 2, or 3 of these genes and is susceptible only to races attacking all 4 genes. Thus, Morye differentiates only 2 races although potentially capable of differentiating 16. Consequently, the race classification has been revised and is based on the reaction of 18 differentials each of which appears to possess 1 rust-conditioning gene (9). Results of the current study are interpreted on the basis of the reaction of the new differentials.

Since the purpose of the surveys was to determine the over-all virulence and distribution of physiologic races, the source of most collections was numerous uredial pustules. Some collections secured early in the season or from sparsely infected plants or fields were of single aecial or uredial origin. The usual procedure was to place 6–8 heavily infected leaves in a glassine envelope or a cork-stoppered glass vial. The leaves kept satisfactorily for several days at cool temperatures. As soon as possible the collection was increased in the greenhouse by inoculating a pot of susceptible plants, usually the Bison variety. Spores secured from this inoculation were stored in glass vials in a refrigerator at 4° C. until the advent of cool fall weather permitted satisfactory physiologic race tests in the greenhouse. In some heavily infested fields it was possible to secure sufficient inoculum for a physiologic race test by collecting the spores on a 4 × 6 in. card, transferring them to glass vials, and storing until physiologic race determinations could be made.

Techniques of inoculation, incubation, and race identification have been described (9). The differential varieties were inoculated with the urediospore collection and the screening effect of each differential was utilized to separate the races in the collection by obtaining subcultures from each susceptible differential for a secondary test. Because all differentials resistant to the original culture should be resistant to the subcultures, only those susceptible to the original collection were used in testing the pathogenicity of the subcultures. This method gave the information essential to the flax breeder, namely, the over-all virulence of the races attacking the various differentials, and assured the detection of races of widest virulence as measured by the number of differentials attacked. It did not give accurate information as to the prevalence or distribution of all races as the less virulent ones may have escaped detection.

RESULTS.—The reactions of the differential flax varieties to races isolated from rust collections made in the North Central States from 1931 to 1951, inclusive, are in Table 1. Because new varieties were added and changes made in the differentials as the study on

[1] Accepted for publication July 31, 1953.
Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, and the North Dakota Agricultural Experiment Station.

[2] Plant Pathologist, Division of Cereal Crops and Diseases.

TABLE 1.—*Reaction of rust differentiating varieties of flax to 34 physiologic races of flax rust collected in the North Central States, 1931 to 1951*

	Reaction of differential variety [a]										
Race no.	Dakota C.I. 1071	Bombay C.I. 42	Stewart C.I. 1072	Koto C.I. 842	Clay C.I. 1188	Akmolinsk C.I. 515	Abyssinian C.I. 701	Wilden C.I. 1193	Williston Brown C.I. 803	Victory A C.I. 1170	No. of homozygous recessive genes
1	R [b]	R	R	R	R	R	R	R	S	R	0
2	R	R	R	R	R	R	R	S	S	R	1
3	R	R	R	R	R	S	R	R	S	R	1
8	R	R	R	R	R	S	S	S	S	R	3
9	R	R	S	R	R	R	R	S	S	R	2
10	R	R	R	R	R	R	R	R	R	R	1
13	R	R	S	R	R	R	R	R	S	R	1
16	R	R	S	R	R	S	S	R	S	R	3
23	R	R	R	R	R	S	S	S	S	R	3
24	R	S	R	S	R	R	R	S	S	R	3
34	R	R	R	R	R	S	R	S	S	R	2
35	R	S	R	R	R	R	R	S	S	R	2
41	R	S	R	R	R	R	R	R	S	R	1
136	R	R	S	R	R	S	R	R	S	R	2
137	R	R	S	R	R	S	R	S	S	R	3
166	S	R	R	S	R	R	R	R	S	R	2
178	S	R	R	R	R	S	R	R	S	R	2
179	S	R	R	R	R	R	R	S	S	R	2
180	S	R	R	R	R	R	R	R	S	R	1
184	R	R	S	S	R	R	R	R	S	R	2
206	R	R	R	S	R	R	R	S	S	R	2
208	R	R	R	S	R	R	R	S	S	S	3
210	R	R	R	S	R	R	R	R	S	R	1
225	R	R	R	R	R	R	R	R	S	S	1
226	R	R	R	S	R	R	R	R	S	S	2
228	R	R	R	S	S	R	R	R	R	R	3
234	S	R	R	R	R	S	R	S	S	R	3
235	R	R	R	S	S	R	R	S	S	R	3
236	S	R	R	S	R	R	R	S	S	R	3
237	S	R	S	R	R	S	R	S	S	R	4
238	S	R	R	S	R	R	R	R	S	S	3
240	R	R	S	R	R	R	R	R	S	S	2
241	S	R	S	R	R	R	R	R	S	R	2
242	S	R	R	S	R	S	R	R	S	R	3

[a] Ottawa 770 (C.I. 355), Cass (C.I. 1182), Polk (C.I. 1191), Birio (C.I. 1085), Kenya (C.I. 709), Leona (C.I. 836) and Bowman (C.I. 1184) are resistant to North American races; Bison (C.I. 389) is susceptible to North American races.
[b] R = not susceptible, reaction types 0, 1, and 2; S = susceptible, reaction types 3 and 4.

physiologic specialization progressed, race identifications made during the different periods are not exactly comparable. Of the 18 differentials used to identify the 1948–1951 races only Stewart, J. W. S., Akmolinsk, Abyssinian, Wilden (Williston Golden), and Williston Brown served to identify the 1931–1934 collections. Bombay was added to the differentials in 1935. When races attacking Koto were discovered in 1942 and Victory A in 1943, the reaction of these varieties to the type uredial culture of each race was determined. While it may not be fully justifiable, it is expedient to consider that all the cultures of a race identified during a period produced the reactions of the type race. For example, all the added differentials were resistant to the type cultures of races 1, 2, and 3 collected in 1931 and it is assumed that the 95 cultures of race 1, the 53 cultures of race 2, and the 51 cultures of race 3 identified during the 1931–1940 period likewise were unable to attack these differentials. However, the type culture of race 24, isolated in 1938, attacked Koto. While the 2 race 24 isolates from the 1939 collections had not been maintained and could not be tested on Koto, Victory A, Dakota (Newland), and Clay it is assumed that they possessed the pathogenicity of the type isolate of race 24.

The number of times each race was isolated is given by periods in Table 2. From 1931 to 1940 most of the rust collections were secured on Bison, then the dominant variety. During this period races 1, 2, and 3, races of narrow virulence, comprised more than 90 per cent of those identified.

Koto was grown extensively from 1942 to 1947 and approximately ¼ of the collections were secured on this variety. During this period races 1, 2, 3, and 210 occurred most frequently. Race 210 differs from race 1 only in that it attacks Koto.

Dakota and Koto were the only rust-susceptible varieties grown extensively from 1948 to 1951, and most of the rust collections were secured on these 2 varieties. Races 166, 180, and 210 predominated and differ from race 1 only in being able to attack Dakota, or Koto, or both.

Studies on the inheritance of rust resistance in flax (6) and on the genetics of pathogenicity in flax rust (5) have indicated a gene for gene relationship between resistance and pathogenicity. For each gene

TABLE 2.—*Prevalence of races of flax rust in the North Central States, 1931-1951*

Race number	Number of times isolated during 1931–1940	1942–1947	1948–1951
1	95	35	5
2	53	26	2
3	51	26	2
8	1	–	–
9	4	2	–
10	1	–	–
13	5	8	1
16	1	–	–
23	1	–	–
24	3	–	–
34	–	4	–
35	2	–	–
41	–	1	–
136	–	1	–
137	–	4	–
166	–	–	67
178	–	–	20
179	–	–	13
180	–	–	50
184	–	2	1
206	–	7	5
208	–	–	1
210	–	27	21
225	–	5	–
226	–	–	1
228	–	–	1
234	–	–	1
235	–	–	1
236	–	–	4
237	–	–	2
238	–	–	1
240	–	1	1
241	–	–	3
242	–	–	1
Total	217	149	204

conditioning resistance in the host there is a specific gene for virulence in the pathogen. Resistance invariably has been dominant whereas virulence, except on Williston Brown, has been recessive. Consequently, the number of pairs of recessive genes demonstrable in each race of flax rust is equal to the number of differentials it attacks, plus or minus 1 depending upon whether Williston Brown is resistant or susceptible.

The proportion of races identified during each period having 0, 1, 2, 3, and 4 pairs of recessive genes for pathogenicity is in Table 3. The decrease in the percentage of isolates having none and the increase in the percentage of those having 2 or more pairs of recessive genes during recent years suggests a tendency for the recently prevailing races to be more widely virulent.

However, this conclusion is not warranted because the 1931–1940 isolates were differentiated by the reaction of 5 or 6 varieties whereas 8 effective varieties were used from 1942 to 1947, and 10 effective varieties were used from 1948 to 1951. Had the 1942–1947 isolates been identified by the differentials in use during the 1931 to 1940 period the percentage of isolates with 0, 1, 2, and 3 pairs of recessive genes would have been 45, 47, 5, and 3 respectively. Based on the reaction of these same differentials 71 per cent of the 1948–1951 isolates would be classed as having 0, 27 per cent as having 1, 1 per cent as having 2, and 1 per cent as having 3 pairs of recessive genes.

A better indication of trends in the prevalence of races having wider virulence is the percentage of isolates that attacked Akmolinsk, Abyssinian, Stewart, Wilden, Bombay, and Williston Brown in each period (Table 4). Since 1930, no variety grown commercially in the North Central States is known to have carried any of the genes conditioning rust reaction in these varieties. Consequently, host screening had no effect on the survival of these genes.

There was a decrease in the percentage of isolates attacking Akmolinsk, Abyssinian, Stewart, Wilden, and Bombay between the periods 1931–1940 and 1948–1951. Isolates attacking Stewart were more numerous from 1942 to 1947 than from 1931 to 1940 because varieties carrying the J.W.S. gene were used in breeding improved flax varieties. One selection carrying this gene was grown in an increase field but was not distributed because of heavy rust infection in 1943. Abyssinian has been resistant to all rust collections made since 1938. The latest isolate to attack Bombay was collected in 1942. Williston Brown, on which virulence is dominant, has been resistant to only 2 isolates, race 10 collected in 1934 and race 228 collected in 1949.

Races attacking Koto and Dakota were discovered in 1942 and 1948, respectively. Although no longer resistant to rust, both varieties continued to be grown because of their desirable agronomic characteristics. From 1948 to 1951 most rust collections were secured on Koto or Dakota and the bulk of surviving races attacked either or both these varieties.

A race attacking Victory A, one of the strains in the mixed variety Victory (7) was isolated in 1943. Although Victory continues to be grown, there has been no significant increase in races attacking the A line.

Clay has been susceptible to 2 isolates, both collected in 1949.

DISCUSSION.—Flax rust has played an important role in the popularity of flax varieties in the North Central

TABLE 3.—*Number of pairs of recessive genes for pathogenicity possessed by isolates of Melampsora lini collected in the North Central States, 1931–1951*

Period isolated	Percentage of isolates having 0 pairs of recessive genes	1 pair of recessive genes	2 pairs of recessive genes	3 pairs of recessive genes	4 pairs of recessive genes
1931-1940	44	50	3	3	0
1942-1947	24	62	11	3	0
1948-1951	2	37	55	5	1

TABLE 4.—*Pathogenicity of isolates on varieties differentiating North American races of Melampsora lini*

Period isolated	Percentage of isolates carrying homozygous recessive genes for Virulence on									Avirulence on Williston Brown
	Akmolinsk	Abyssinian	Stewart	Wilden	Bombay	Koto	Dakota	Victory	Clay	
1931-1940	25	1	5	30	2	1	0	0	0	1
1942-1947	23	0	12	29	1	24	0	4	0	0
1948-1951	12	0	3	14	0	51	79	2	1	1

States between 1931 and 1951. Likewise, the varieties of flax grown largely determined the prevalence of the different races of rust. Since this rust attacks only species of *Linum* (flax), the survival of a particular race depends upon the continued production of varieties susceptible to it. Races unable to attack current commercial varieties disappear while those attacking the dominant varieties tend to increase.

Bison was released as a rust-resistant variety in 1926 but it has been susceptible to all collections of flax rust made in North America since 1931, when physiologic race studies were started. As late as 1935, Dillman and Stoa (2) stated that Bison, Buda, and Redwing are rarely injured by rust, although not immune. Field notes recording the rust reaction of flax varieties during the late 1920's indicate that the resistant reading on Bison was based on fewer infections than on N.D.R. 114 and other rust-susceptible varieties then grown but not on the production of a resistant type of pustule. This suggests that Bison was resistant to all but a few of the races present at that time. As Bison became the dominant variety, races unable to attack it died out and those attacking it increased. The fact that Bison is immune from some races in southwestern Australia (13) supports this hypothesis. Races to which Bison is resistant are not available for study in North America and the nature of rust resistance in Bison and of pathogenicity on it are not known.

The history of rust on Koto, one of the varieties developed as a rust resistant replacement for Bison, parallels that of Bison. Koto was free of rust during several years of testing at experiment stations throughout the North Central States. In 1942, however, it rusted while being increased for distribution. By 1946, a majority of field collections contained races attacking Koto.

Dakota, a variety carrying the Newland gene for rust reaction, was distributed in 1945. Varieties possessing this gene had been immune from rust for more than 30 years. Late in the season of 1948, a light infestation of rust was found on Dakota in the Red River Valley of Minnesota and North Dakota. The new races spread north and south more rapidly than westward. In 1949, rust was observed on Dakota in a fan shaped area extending from a few miles west of the southeast corner of North Dakota to ⅔ across the northern boundary of the state (8). By 1950, the new races had spread over the northeastern ⅔ of the state, and in 1951 they were found in the southwestern portion. With the spread of the new races Dakota was heavily damaged in 1949 and 1950, and in 1951 loss due to rust approximated 3,000,000 bushels. By 1952 the rust resistant varieties B5128, Marine, Redwood, Rocket, and Sheyenne, had largely replaced Dakota.

Many of the genes for rust reaction in flax condition either high resistance or complete susceptibility. A flax variety carrying 1 rust-conditioning gene is susceptible to all races carrying the specific gene for virulence which overcomes or renders the host's rust-conditioning gene ineffective, and is resistant to all races not carrying this gene. Virulence is recessive except on Williston Brown. Therefore, races having the widest host range possess the smallest complement of dominant genes and should display the least vigor if vigor is a function of the number of dominant genes present.

No relation between vigor and the number of dominant genes carried by a race has been observed in greenhouse tests. Race 22, which possesses at least 20 pairs of recessive genes for pathogenicity, apparently sporulates as abundantly on Bison as does race 1 which carries none. Nevertheless, under field conditions, vigor in a race may have some survival value. If rapid spore production, rapid and vigorous spore germination and infection, wide temperature range for spore germination and infection, or tolerance to high temperatures were associated with the number of dominant genes, races attacking only the current commercial varieties would have a survival advantage in the North Central States.

Ninety-two per cent of the isolates from 1931 to 1940, when Bison was the leading variety, were races 1, 2, and 3. Race 1 carries 0 pairs of recessive genes and races 2 and 3 each carry 1 pair. From 1942 to 1947, when Koto was grown widely, 77 per cent of the isolates were races 1, 2, 3, and 210. Race 210 differs from race 1 only in its ability to attack Koto. From 1948 to 1951, when Koto and Dakota were leading varieties, 68 per cent of the isolates were races 166, 180, and 210. These races differ from race 1 only in attacking either Koto, or Dakota, or both. The dominant races during each of these 3 periods were those carrying the least number of recessive genes compatible with survival. The proportion of isolates carrying genes for virulence having no survival value decreased in each successive period. In the North Central States, where hybridization and segregation occur, heterosis may be a factor in the prevalence of races.

Abyssinian and Bombay have been resistant to all

races tested since 1938 and 1942, respectively, and there has been a continued decrease in the percentage of races attacking the differential varieties possessing rust-conditioning genes that are not found in commercial varieties. This does not necessarily indicate that these races are disappearing. Resistance in these varieties is dominant and their reaction to races heterozygous for the avirulent gene is identical with their reaction to races homozygous for these genes. Consequently, it is not possible to identify races carrying latent genes for virulence. The extensive culture of varieties dependent for resistance on these genes might result in the screening out of nonvirulent races and a rapid build-up of races virulent on these varieties. This appears to have occurred with the Koto and Dakota varieties.

SUMMARY

In the North Central States races of flax rust attacking widely grown varieties increase; races unable to do so, decrease. The predominant races have been those carrying the smallest number of recessive (virulent) genes that permit survival.

Bison, released as a rust-resistant variety in 1926, was susceptible to each of the 570 races isolated from field collections of flax rust during the period 1931–1951, inclusive. From 1931 to 1940, when Bison was the dominant variety, 92 per cent of the isolates were of races 1, 2, and 3. Race 1 carries none, and races 2 and 3 each carry a single pair of recessive genes for pathogenicity on the rust-differentiating varieties.

Koto was the most widely grown rust-susceptible variety from 1942 to 1947, and races 1, 2, 3, and 210 were the dominant races. Ability to attack Koto differentiates race 210 from race 1.

Koto and Dakota, the only rust-susceptible varieties that were widely grown from 1948 to 1951, were attacked by 95 per cent of the isolates made during these years. Races 166, 180, and 210 differ from race 1 only in their ability to attack Koto, Dakota, or both.

During each period there was a decrease in the percentage of isolates that were virulent on differential varieties having rust-conditioning genes that were not present in commercial or experimental varieties. This indicates that in the North Central States the possession of a maximum number of dominant genes may have survival value.

State College Station
Fargo, North Dakota

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EFFECT OF SPRAYING ON INCIDENCE OF DISEASES AND YIELDS OF MUSCADINE GRAPES [1]

E. S. Luttrell and M. M. Murphy

In contrast with bunch grapes, muscadine grapes are not subject to any disastrous diseases. Since it is possible to produce a satisfactory crop without attempting to control diseases, spraying is generally considered unnecessary. However, an appreciable amount of the crop is lost to berry rots each year, and in some years diseases appear to be of such severity that increases in yields might be expected from their control. In spite of negative results reported previously (2), further tests were made at the Georgia Experiment Station during 1946-1951 to determine the percentage of the crop lost to diseases, the increases in yields that might be obtained from spraying, and the period during which sprays are most effective.

THE DISEASES.—Relatively few diseases occur on muscadines, and most of these differ in symptomatology, etiology, and importance from those of bunch grapes.

Black rot (*Guignardia bidwellii f. muscadinii* Lutt.) is primarily a disease of the vegetative parts, causing only scabs and cankers on the berries of the most susceptible varieties (2, 4). Althought leaf spotting may be heavy, it apparently does not affect the yield or vigor of the vines to any serious extent. Berry cankers may lower the quality of the crop, but quantitative losses are negligible.

Angular leaf spot, caused by *Mycosphaerella angulata* W. A. Jenks. (1), affects only the foliage, and at Experiment, Georgia, does not become severe until after harvest. In some localities, particularly under poorer growing conditions, it may develop earlier. However, it usually does not appear to affect the current season's crop, and any effect of premature defoliation on vigor of the vines would probably not be great enough to justify the expense of control measures.

The most important disease is bitter rot, caused by *Melanconium fuligineum* (Scrib. & Viala) Cav. *M. fuligineum* occasionally causes a flecking of young leaves and stems (5). A similar fleck of sepals and blight of individual flower buds also may be attributable to this fungus. It is most serious, however, as a cause of berry rot and pedicel blight. Bitter rot first develops on a few of the young green berries. Olive-brown lesions appear on the berries, and the infected tissue soon becomes densely speckled with black acervuli. The berry remains firm and ultimately shrivels to a hard, dry, black mummy. The disease spreads rapidly as the berries approach maturity. On the ripening berries it causes a soft rot. The rot usually appears first around the pedicel and spreads uniformly down the sides until the whole berry is involved. The skin of purple varieties shows a bleached pink discoloration. A crust of black, slimy acervuli forms over the surface of the rotted berry, and the berry shrivels to form a mummy. The berries may drop in any stage of the rot, or the mummies may cling to the vines.

A blight of the pedicels seems to be another phase of bitter rot. The infected pedicels show an indefinite brownish discoloration, shrivel, and break off. The berries shrivel slightly and drop off singly or in small clusters clinging to the shriveled pedicels. Most of these berries sooner or later develop symptoms of bitter rot. Although no inoculations have been made, pedicel blight is attributed to *Melanconium fuligineum* for the following reasons. This fungus is commonly isolated from incipient lesions on pedicels. Usually bitter rot mummies are present in the clusters affected with pedicel blight, the lesions then appearing on branches of the peduncle where pedicels of infected berries or dead pedicels are attached. The fact that infection of the berries usually takes place at the pedicel end suggests that the fungus enters through the pedicels. This appears to be the means by which the fungus commonly spreads from berry to berry in the cluster, although it may spread by direct contact between healthy and infected berries. *M. fuligineum* fruits on the dead pedicels. The acervuli persist on the dead pedicels and mummies left clinging to the vines well into the next growing season. These together with acervuli on fallen mummies furnish the inoculum for the following year. No perfect stage of the fungus has been found.

Macrophoma ripe rot, although second in importance to bitter rot, causes little damage to the crop. It appears on the ripening berries as small, circular, slightly depressed, brown or tan spots. A soft rot often develops from the primary lesions and spreads irregularly over the berry. The surface is dotted with pycnidia of the causal fungus. The rotted berries are reduced to dry, hollow shells on which the fungus overwinters in the pycnidial stage. The causal fungus is *Botryosphaeria ribis* (Tode ex Fr.) Gross. & Dug. (3).

Traces of a ripe rot caused by *Glomerella cingulata* (Ston.) Spauld. & Schrenk. have been found in some years. It is a soft rot characterized by the presence of the orange acervuli of the causal fungus on the surface of the lesions.

EXPERIMENTAL WORK AND RESULTS.—*Methods.*—Tests were made in an acre muscadine vineyard of the Hunt variety. Vines available for this purpose were

[1] Accepted for publication August 1, 1953.
Paper No. 243, Journal Series, Georgia Experiment Station.
We are indebted to Dr. Mary Speirs, Georgia Experiment Station, and Dr. J. A. Rigney and Mr. Sidney Weiner, Department of Experimental Statistics, North Carolina State College of Agriculture and Engineering, for their assistance in the statistical analysis of the data.

TABLE 1.—*Effect on yields of spraying muscadine grapes with 8-8-100 Bordeaux mixture for control of diseases*

Year	Treatments I Control no spray Yield	II Pre-blossom 3 sprays Yield	II Pre-blossom 3 sprays Difference[a]	III On green berries 3 sprays Yield	III On green berries 3 sprays Difference	IV On ripening berries 2 sprays Yield	IV On ripening berries 2 sprays Difference	V Complete 8 sprays Yield	V Complete 8 sprays Difference
	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds
1946	60	66	+ 6	74	+14	68	+ 8	103	+43
1947	69	80	+11	78	+ 9	89	+20	96	+27
1948	108	85	—23	123	+15	115	+ 7	102	— 6
1949	24	23	— 1	31	+ 7	33	+ 9	36	+12
1950	90	62	—28	114	+24	105	+15	82	— 8
Five year mean	70	63	— 7	84	+14	82	+12	84	+14

[a] Least difference required for significance between effects of treatments on yields in different years is 14 lb. (odds of 19 to 1) and 18 lb. (odds of 99 to 1).

divided into 5 groups of 8 vines each. The vines were matched on the basis of average annual yields for the 5-year period (1941-45) preceding the test and distributed so that high and low yielding vines occurred in all groups. The average annual yield per vine for each of the resulting groups was approximately 56 pounds. The groups received the following treatments: I—control, no spray; II—3 pre-blossom sprays at 2-week intervals beginning about the middle of April when the new shoots were 4-5 in. long; III—3 sprays on the green berries at 2-week intervals beginning in the latter half of June soon after the berries were set; IV—2 sprays on the ripening berries at 2-week intervals beginning 2 weeks after the last spray in treatment III; V— complete spray, a combination of the 8 sprays in treatments II, III, and IV. An 8-8-100 Bordeaux mixture was used for all sprays except the first of the pre-blossom sprays in which a 4-6-100 Bordeaux mixture was substituted to reduce injury to the young shoots. This test was started in 1946 and continued through 1950. In order to determine any cumulative effect of spraying, each group received the same treatment throughout the period.

In 1950 2 additional treatments were included in the test. One consisted of 3 sprays of Bordeaux mixture at 1-month intervals beginning soon after the berries were set; the other of 3 sprays of zineb (Dithane Z-78, 1½ lbs. to 100 gal. water[2]) at the same times.

In 1951 the test was reorganized. The vines were divided into 6 groups of nine vines each which received the following treatments: I—control, no spray; II—3 sprays of Bordeaux mixture at 2-week intervals; III—3 sprays of Bordeaux mixture at 1-month intervals; IV—5 sprays of Bordeaux mixture at 2-week intervals; V—3 sprays of zineb at 2-week intervals; VI—5 sprays of zineb at 2-week intervals. The first spray in each treatment was applied soon after the berries were set.

Observations on incidence of berry diseases were made on 3 vines in each treatment in 1946, on all vines in each treatment in 1950, and on all vines in 3 treatments in 1951. All of the berries that dropped beneath each vine were collected each week until harvest time, counted, and classified according to cause of shelling as follows: 1) Sound—no apparent cause. Some physiological shelling of sound berries occurs on all varieties. 2) Unknown—berries covered by molds or otherwise unclassifiable. Some were destroyed by bees or birds. 3) Curculio—some shelling of young green berries was caused by the insect *Craponius inaequalis* (Say.). 4) Bitter rot. 5) Pedicel blight—considered only a phase of bitter rot and combined with bitter rot to obtain a complete estimate of the damage caused by this disease. 6) Black rot. 7) Macrophoma ripe rot. Losses were expressed as percentages of the potential yield, which was obtained by adding the number of pounds harvested and the calculated number of pounds that dropped from the vines prior to harvest.

Data on yields were treated statistically by analysis of variance (7). The non-randomization of the treatments on the same vines from year to year required the use of a modified estimate of variance for evaluating treatment effects. Approximations for error term and number of degrees of freedom were determined by Satterthwaite's (6) formula.

Effect of spraying on incidence of diseases.—Data on incidence of berry diseases and their control by spraying with Bordeaux mixture for 1946 and 1950 are in Table 2. In Table 3 are data on the comparative effects of Bordeaux mixture and zineb on disease incidence in 1950 and 1951. Data on unsprayed control vines indicated that in 1946, 1950, and 1951 the percentage of the crop shelled prior to harvest varied from 20–31 per cent. Bitter rot (including pedicel blight) was the only important disease, accounting for losses of 13–26 per cent of the crop. Percentage losses in other categories varied as follows: Macrophoma ripe rot 0-2.7, black rot 0-0.2, curculio 0-0.2, unknown 0.7-6.3, sound 0.2-6.0 per cent. Only the sprays applied to the berries produced reductions in disease losses. Of these, 3 sprays of Bordeaux mixture applied to the green berries at 2-week intervals

[2] Furnished by Rohm & Haas Company, Washington Square, Philadelphia 5, Pennsylvania.

TABLE 2.—*Effect of spraying muscadine grapes with 8-8-100 Bordeaux mixture on incidence of berry diseases*

Treatment	Year	Potential yield	Actual yield	Berries shelled	Cause of shelling	
					Bitter rot	Pedicel blight
		pounds	pounds	per cent	per cent	per cent
I. Control no spray	1946	106	76	28.1	11.4	6.0
	1950	113	90	20.3	10.0	2.5
II. Pre-blossom 3 sprays	1946	111	77	30.4	10.0	8.7
	1950	80	62	20.3	6.3	3.7
III. On green berries 3 sprays	1946	115	100	13.1	5.3	4.0
	1950	126	114	9.5	3.6	1.9
IV. On ripening berries 2 sprays	1946	102	78	23.7	9.3	6.0
	1950	122	105	13.9	6.2	2.1
V. Complete 8 sprays	1946	117	97	17.2	2.9	9.3
	1950	94	82	12.6	2.0	4.2

(Treatment III) were the most effective, reducing shelling and incidence of bitter rot by approximately one half (Table 2). Three sprays of Bordeaux mixture applied to the berries at 1-month intervals in 1950 were equally as effective as 3 sprays at 2-week intervals (Table 3). Three sprays of zineb at 1-month intervals in 1950 had little effect on diseases (Table 3). When in 1951 the intervals were reduced to 2-weeks and 5 sprays were applied, results with zineb were as good as or better than those with an equal number of sprays of Bordeaux mixture in reducing shelling and incidence of bitter rot. However, zineb did not seem effective against Macrophoma ripe rot.

Effect of spraying on yields.—Table 1 gives the data on the effect of the various spray treatments with Bordeaux mixture on yields. The 3 pre-blossom sprays did not increase yields. In fact, they caused significant reductions in 1948 and 1950 and a slight reduction in average annual yield for the 5-year period. Sprays applied to the berries produced varying increases. The most consistent results were obtained with 3 sprays on the green berries at 2-week intervals (Treatment III). With this treatment significant increases over the control were obtained in 3 years and the average annual yield was increased by 20 per cent. Two sprays applied to the ripening berries (Treatment IV) were about as effective. The complete schedule of 8 sprays (Treatment V) also produced an average annual increase of 20 per cent and significant increases in 2 years. In 1948 and 1950, however, results reflected the injurious effect of the 3 pre-blossom sprays included in this treatment, and decreases in yields were recorded.

In 1950 (Table 3) 3 sprays of Bordeaux mixture at 1-month intervals beginning soon after the berries were set gave approximately the same results as 3 sprays of Bordeaux mixture at 2-week intervals. Three sprays of zineb were not effective.

A severe freeze in November 1950 before the vines were completely dormant damaged the vines to such an extent that yield records for 1951 were of no value and the vineyard was ruined for further experimental work in the immediate future. Yields for 1951, therefore, are not reported except incidentally in Table 3 for 3 treatments for which data on disease incidence were collected. During the extremely dry 1951 season, 5 applications of Bordeaux mixture on the berries left a heavy residue and it was apparent from the shriveled berries that it caused considerable injury.

TABLE 3.—*Comparative effect of spraying with 8-8-100 Bordeaux mixture and zineb on incidence of berry diseases and yields of muscadine grapes*

Years and treatments	Potential yield	Actual yield	Difference from control	Berries shelled	Cause of shelling	
					Bitter rot	Pedicel blight
1950	pounds	pounds	pounds	per cent	per cent	per cent
Control	113	90	——	20.3	10.0	2.5
Bordeaux—3 sprays at 2 week intervals	126	114	+24	9.5	3.6	1.9
Bordeaux—3 sprays at 1 month intervals	121	109	+19	9.7	4.0	1.9
Zineb—3 sprays at 1 month intervals	98	81	— 9	17.2	7.1	2.6
1951						
Control	72	50	——	30.7	20.4	5.2
Bordeaux—5 sprays at 2 week intervals	43	35	—15	18.9	10.8	5.1
Zineb—5 sprays at 2 week intervals	55	45	— 5	17.7	7.1	4.2

TABLE 4.—*Variations in percentage of berries shelled among varieties of muscadine grapes*

Variety	Year	Potential yield	Actual yield	Berries shelled
		pounds	pounds	per cent
Creek	1945	42	25	40.5
	1946	101	72	28.7
Dawn	1945	40	32	20.0
	1946	53	36	32.0
Dulcet	1945	50	38	24.0
	1946	74	60	18.9
Hunt	1945	79	52	34.0
	1946	79	52	34.0
Irene	1945	37	28	24.3
	1946	82	72	12.2
Lucida	1945	74	11	85.1
	1946	64	23	64.1
November	1945	36	28	22.2
	1946	81	67	17.3
Scuppernong	1945	35	24	31.4
	1946	79	60	24.1
Stuckey	1945	53	25	52.8
	1946	27	15	44.4
Thomas	1945	26	23	11.6
	1946	89	78	12.4
Yuga	1945	77	53	32.5
	1946	44	32	27.3
6-17	1945	88	77	12.5
	1946	120	112	06.7

Variation in shelling among muscadine varieties.—In 1945 and 1946 all berries that dropped beneath 2 vines of each variety in the Georgia Experiment Station vineyard were counted and classified. Data in Table 4 on percentage of berries shelled from each variety afford a comparison of crop losses on other varieties with those on the Hunt variety, which has been studied more extensively. Shelling on most varieties was about the same as that on the Hunt. Shelling was excessive on several varieties, particularly the Lucida. The greater amount of shelling on these varieties was the result of increased shelling of sound berries. This is attributed to physiologic causes. Some varieties apparently have an inherent tendency toward physiologic shelling, although this tendency may be modified by environment. Apparently there is less shelling of this nature on the Lucida when it is growing on poorer soils.

The relative importance of diseases on most varieties was approximately the same as on the Hunt. Bitter rot was the only serious disease. Although Thomas was more susceptible to Macrophoma ripe rot, the disease was of minor importance even on this variety. Only 2 varieties showed strikingly less shelling. These were the Thomas and the unnamed variety No. 6-17 (sometimes referred to unofficially as "Big Boy"). These varieties appeared to have a degree of resistance to bitter rot. They also are resistant to black rot (2).

DISCUSSION.—The variations in yields among vines and for individual vines in different years were extreme. A great deal of variation results from the effect of the previous season's yield on the vines, each vine following an individual and irregular cycle over a period of years. Weather conditions, such as late spring freezes and rain during blooming time, also produce wide variation in yields from year to year aside from their effect on the development of diseases. Because of this variation, the overall effects of treatments were not clear cut. Although treatment III produced significant increases in 3 years and treatments IV and V in 2 years, an analysis of overall treatment effects for the 5-year period, using Satterthwaite's (6) approximation for error term, indicated a probability of only about 4 to 1 that spraying will increase yields. Consequently, the only conclusion that can be safely drawn is that spraying with Bordeaux mixture has not been demonstrated to be of value in the production of muscadine grapes.

A consideration of data on disease incidence supports this conclusion. Data in Tables 2 and 3 indicate that losses to shelling, not all of which were attributable to diseases, were only 20–31 per cent. Although these figures do not include losses resulting from cull, it seems improbable that average annual increases in excess of about 25 per cent could be expected from control of berry diseases by spraying.

The data show certain trends which might be considered in planning future work. It is apparent that diseases of muscadines present a different problem from that presented by diseases of bunch grapes. On muscadines black rot is of little importance on the berries, and control of this disease on the foliage has little effect on the subsequent development of berry rots. It seems, therefore, that pre-blossom sprays on muscadines are unnecessary. Certainly a less injurious material than Bordeaux mixture would have to be used. With muscadines the sprays applied to the berries are of greatest importance, since the only serious disease is bitter rot and this is important only on the berries. With Bordeaux mixture the most effective schedule would be 3 sprays applied to the berries. Additional sprays with this material build up heavy and objectionable residues on the berries and may cause injury.

Although the 1950 freeze intervened before a comparison of zineb and Bordeaux mixture could be carried out, data on disease incidence in 1951 indicate that 5 sprays of zineb at 2-week intervals controlled diseases as effectively as Bordeaux mixture. Zineb has the advantages of not injuring the vines and also of producing no objectionable residue. The latter feature is important since bitter rot is serious on the maturing berries and it is necessary to keep them covered until harvest time.

SUMMARY

Although black rot and angular leaf spot attack the foliage of muscadine grapes, they apparently have little direct effect on yields. The most important diseases are berry rots, of which bitter rot is the most serious. Shelling of berries on the Hunt varied from 20 to 31 per cent. Losses to bitter rot varied from 13 to 26 per cent. Combined losses to all other berry diseases, including black rot, Macrophoma ripe rot, and Glomerella ripe rot, and to curculio did not ex-

ceed 3 per cent.

In tests with an 8-8-100 Bordeaux mixture on vines of the Hunt variety pre-blossom sprays did not increase yields on the average and in some years decreased yields. The most effective spray schedule was 3 sprays applied to the berries at 2-week intervals beginning soon after the berries were set. This treatment halved the amount of shelling and bitter rot and produced over a 5-year period an average annual increase in yield of 20 per cent. Differences between sprayed and control vines are not considered sufficient to justify a recommendation of spraying for the control of muscadine grape diseases.

In 1 year, 5 sprays of zineb (Dithane Z-78) on the berries at 2-week intervals were as effective as sprays of Bordeaux mixture in reducing shelling and incidence of bitter rot, but no adequate data on its effect on yields were obtained.

GEORGIA EXPERIMENT STATION
EXPERIMENT, GEORGIA

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LINEAR GROWTH OF SCLEROTIUM BATATICOLA THROUGH SOIL[1]

Don C. Norton

During recent years, charcoal rot, caused by *Sclerotium bataticola* Taub., has become the major disease of guayule in Texas. Diseased plants may be scattered throughout a field and there appears to be no definite pattern as to the spread of the pathogen through the soil. The pycnidial form of the fungus, *Macrophomina phaseoli* (Maubl.) Ashby, has never been found on guayule and there is no evidence that aerial infection occurs by pycnidiospores from other plants. The pycnidial stage of the isolate used was not obtained, even by the method of Luttrell (9).

There have been indications that the charcoal rot fungus is greatly inhibited by other soil microorganisms. Livingston (8) reported that there was practically no seedling blight of sorghum in infested chloropicrin-treated soil as compared with much infection in infested steam-sterilized soil. Semeniuk (14), with corn, and Luttrell and Garren (10), with beans, reported little or no infection by *S. bataticola* in unsterilized soil. Vasudeva and Sikka (20) reported a dissolving effect of *Rhizoctonia bataticola* (Taub.) Butler by *Aspergillus niger* and *Trichoderma lignorum* in culture.

In the United States, charcoal rot usually is most serious during hot, dry weather. Application of water almost completely controls the disease even though high temperatures prevail (8, 11, 12). On agar media, the optimum temperature for the growth of *S. bataticola* is in the vicinity of 30°–35°C. (7, 8, 15, 18, 19). In general, this is from 5 to 10 degrees above the optimum of many of the common soil inhabiting fungi.

The present work was undertaken to investigate the effect of temperature and moisture on the linear growth rate of the charcoal rot fungus through soil.

MATERIALS AND METHODS.—The fungus isolate was obtained from diseased guayule during the summer of 1951 and appeared to be representative of the numerous isolates obtained.

The tube method of Ryan et al (13) was employed for the linear growth studies of the charcoal rot fungus on yeast extract-dextrose agar. The length of the agar surface was 25 cm. The medium contained 2.5 gm. K_2HPO_4, 6.0 gm. yeast extract, 20.0 gm. glucose and 20.0 gm. agar per liter. Measurements were recorded daily for a period of 10 days, or until the fungus had completely covered the agar surface. The tubes were kept in the dark at 25°, 30°, 35°, and 40°C. The average linear growth in 3 tubes at each temperature was taken as the final reading.

The soil was a Duval fine sandy loam with a pH of 7.7. This supports an excellent growth of guayule but is also a soil type in which loss from charcoal rot has been extremely high (11).

For studies of the growth of the fungus through soil, a modified method of Eaton et al (5) was adopted as being best for maintaining the most constant soil moisture level. Glass tubes, approximately 60 cm. in length, with an internal diameter of 1.5 cm., and sealed at one end, were filled to a depth of 40 cm. with soil. In all cases, the soil was first air dried, sieved through a 10-mesh screen, and then mixed with the correct amount of water to give it the desired moisture content. The moist soil was then added to the tubes. If sterilization was required, the tubes and soil were autoclaved for 2 hours at this point. The fungus was added, either with or without a food base. Where no food

[1] Accepted for publication August 1, 1953.

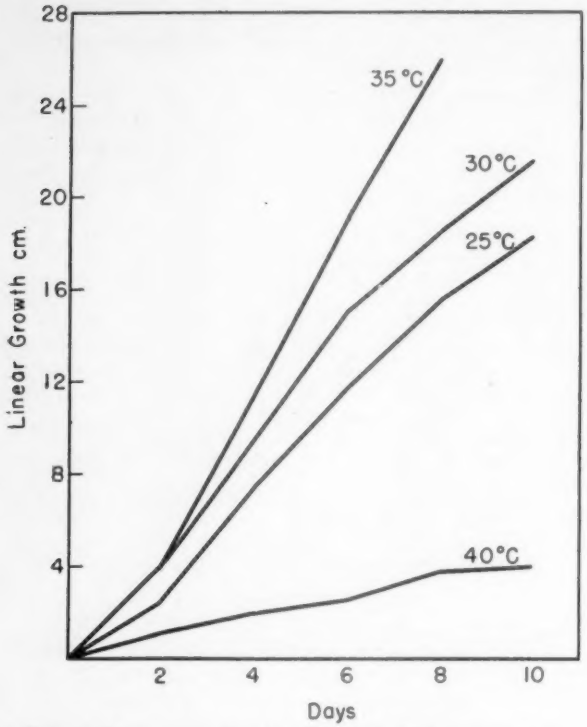

FIG. 1. Linear growth rate of *S. bataticola* on yeast extract-dextrose agar at various temperatures.

base was used, the charcoal rot fungus was cultured on nutrient solution for 3 days. Sections, 1.4 cm. in diameter, were cut from the margin of the mycelial mats, washed thoroughly with sterile water, and added to the tubes. Where a food base was desired, either 1 tablespoonful of ground sterilized guayule roots, on which the charcoal rot fungus had been growing for 5 days, or an agar disc., 1.4 cm. in diameter, taken from the margin of a 2-day-old yeast extract-dextrose agar culture, was added to the tubes. After addition of the fungus, the following were added in order: 5 cm. of sterile quartz sand, 5 cm. of sterile soil to which 3 ml. of sterile water was later added, sterile quartz sand nearly to fill the tubes, and finally cotton plugs. The same temperatures as used in the growth studies on agar were used in this and all subsequent tests. The soil moisture levels used were 5, 10, 15, and 20 per cent moisture content. This corresponds to 18.5, 37.0, 55.6, and 74.1 per cent of saturation for this particular soil. For each series of tests, 3 tubes were used at each temperature for every soil moisture level. The farthest point of growth was recorded daily, for a period of 10 days, by observing the mycelial growth in the tubes by means of a dissecting microscope. Autoclaving sometimes caused changes in the soil moisture level beyond that desired. If the change was greater than 1 per cent, the tube was discarded.

In one experiment, the Conn (4) modification of the Rossi-Cholodny technique was used, with the exception that after filling the tumblers with soil of the desired moisture content, the soil was covered with a layer of sterile dry quartz sand, which in turn was covered with a layer of soil at field capacity. The same temperature and soil moisture levels were used as in the soil tube experiment with the exception that 0 and 2½ per cent soil moisture content were added and the 20 per cent was omitted. Three tumblers, each containing 4 slides, were used for each temperature and moisture level. A disc, 0.8 cm. in diameter, taken from the marginal growth of a 2-day-old culture growing on yeast extract-dextrose agar, was placed on the inner face of each slide. Following a 5-day incubation period, the core of soil was removed from the tumblers and the slides were carefully removed, fixed, and stained with erythrosin.

RESULTS.—The optimum temperature for the linear growth rate of *Sclerotium bataticola* on yeast extract-dextrose agar was 35°C., although there was abundant growth at 30° and 25°C. (Fig. 1). In general, the optimum agrees with that obtained with isolates used by Uppal et al (18), Tompkins and Gardner (15), and Livingston (8).

In the absence of a food base in sterile soil, the optimum linear growth of the organism was obtained

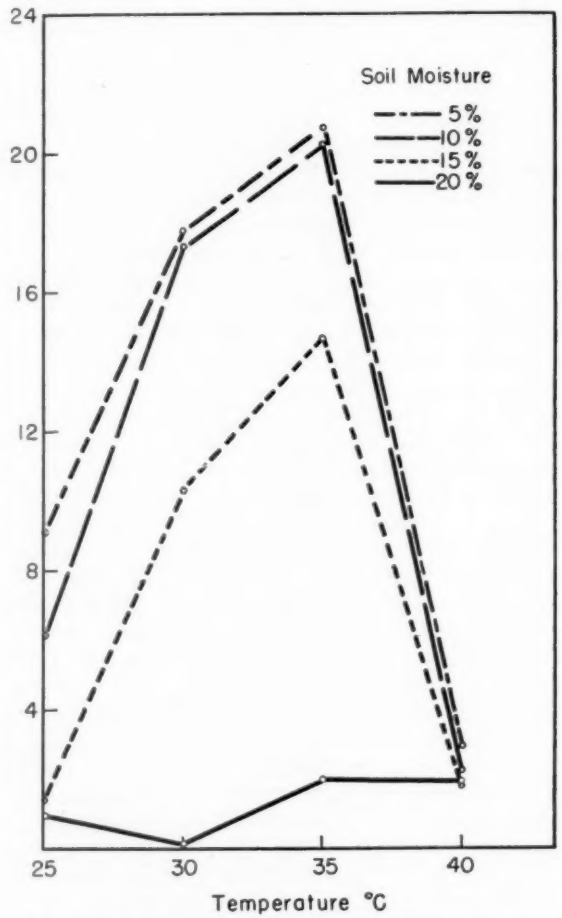

FIG. 2. Total 10 day growth of *S. bataticola* through columns of sterile soil at various temperature and moisture levels.

at 35°C. at the 5 per cent soil moisture level (Fig. 2). At all temperatures employed, growth was only slightly more at the 5 per cent than the 10 per cent soil moisture level. Soil with a 20 per cent moisture content approached the consistency of mud and all growth that occurred was through air pockets. At all temperatures, growth was faster and much more abundant on an agar medium than in sterile soil. In nearly all cases, the faster the growth, the more abundant and wider were the hyphae. At 25°C., the hyphae were few in number. In all instances in which a sterile substrate was used, growth was fairly uniform over the 10-day period.

The only growth that occurred when mycelium was placed into unsterilized soil occurred at 35°. However, this growth was slight, a total of 0.2 cm., 0.1 cm., and 0.3 cm. occurring over a 10-day period at 5, 10, and 15 per cent soil moisture content respectively.

In no case was there growth into the unsterilized soil where a guayule food base was used.

At all temperatures, there was limited growth into unsterilized soil when a yeast extract-dextrose agar food base was used (Table 1). With the exceptions of the 40°C. temperature and the 20 per cent soil moisture series, there was little difference in the amount of growth that occurred.

When unsterilized soil was used in tubes, all observable growth into the soil occurred by the end of the third day of the experiment. Growth always occurred for a few centimeters into the sterile quartz sand whether the soil was sterilized or not.

For comparison, the growth rate of *Rhizoctonia solani* Kuehn was measured in unsterilized soil with 5 per cent moisture at 30°C. using a yeast extract-dextrose agar food base. Growth was fairly uniform over a period of 10 days, reaching a total of 15.5 cm. in that period. This was faster than that obtained by Blair (3).

In all soil experiments except those in which a guayule food base was used, it was noted that although no mature sclerotia were introduced into the tubes, many had formed within 24 hours in the initial mycelial mass. However, in no case were sclerotia seen to form in the soil itself or on the walls of the tubing, beyond a distance of 0.2 cm. from the initial source.

Results of the growth studies using the Rossi-Cholodny technique were somewhat uncertain as hyphae did not adhere continuously to the glass slide. As far as could be ascertained, there was no growth in unsterilized soil beyond 2.1 cm. from the food base. However, in most cases, total growth followed the same pattern as in the tubes (Table 1). Growth at 0 per cent soil moisture was slight while that at 2½ per cent was approximately the same as that at the remaining soil moisture levels. With the exception of the tumblers placed at 40°C., growth in the sterile soil extended the entire length of the slide.

On many of the slides buried in unsterilized soil, a symbiotic relationship between *S. bataticola* and other fungi was noted. Whether or not this was parasitism has not been ascertained. Lysis of hyphae by bacteria was observed in a few cases. However, in most observations, the aforementioned phenomena were not found.

TABLE 1.—*Total growth of Sclerotium bataticola from a yeast extract-dextrose agar food base through unsterilized soil after 10 days*

Soil moisture	Temperature °C. 25°	30°	35°	40°
Per cent	cm.	cm.	cm.	cm.
5	1.3	1.9	1.8	0.1
10	0.6	1.6	1.7	0.0
15	1.1	1.6	1.7	0.0
20	0.1	0.0	0.0	0.0

DISCUSSION.—The demonstration of the inability of *Sclerotium bataticola* to grow extensively through the soil supports field and greenhouse observations of other workers. From the results of inoculation experiments on sorghum, Livingston (8) concluded that the charcoal rot organism is greatly inhibited by other soil microorganisms. More recently, Luttrell and Garren (10) found that there was little infection of beans inoculated with the charcoal rot fungus in unsterilized soil. The writer has experienced great difficulty in obtaining infection when guayule plants were inoculated in chloropicrin-treated soil. In addition, all attempts to isolate the causal organism from soil in the field in the immediate vicinity of the roots have failed.

It was reported earlier (8, 11, 12) that water will prevent or almost entirely eliminate losses from charcoal rot. This is contradictory to observations by Uppal et al (18) in India where losses from this fungus are greatest following periods of heavy rains. With guayule, charcoal rot is a problem only during hot, dry weather, during which time the plants are under severe water stress. Andrus (1) and Tompkins and Gardner (15) found that the high temperatures favorable for growth of the charcoal rot fungus are unfavorable for the development of the host. Livingston (8) found that low soil moisture retarded growth of sorghum and favored development of seedling blight and stalk rot. Since *S. bataticola* is strongly inhibited by the soil microflora over a wide range of soil temperature and moisture levels, it would seem that the hot, dry weather is of greater importance in reducing the vigor of the host than in favoring the growth of the charcoal rot fungus through the soil.

The most growth through unsterilized soil occurred where a food base with 2 per cent sugar was used. This supply of nutrient is far greater than that existing in the soil itself. The total organic matter content of the soil used was only 0.43 per cent. Traub and Slattery (16) and Traub et al (17) found that the free sugars in guayule roots varied from season to season. Benedict et al (2) reported little variation in the free sugar content during periods of alternating high and low water stress. In all 3 aforementioned papers, it was reported that the free sugar content in guayule roots was always under 1 per cent, on a dry

weight basis. Whether or not sugar is the limiting factor in the amount of growth made into unsterilized soil, it is highly improbable that a richer substrate than the yeast extract-dextrose agar used occurs naturally in soils.

The inability of the mycelium of the charcoal rot fungus to compete with the soil microflora might be due to the great conversion of the substrate to sclerotia, with the result that little competitive mycelium is formed. Of course, in pure culture, a continuous substrate is available for mycelial production. Even though the growth of *S. bataticola* in pure culture is abundant over wide temperatures and soil moisture levels, this is of little value in the spread of the organism through unsterilized soil. Once a plant has been subjected to a severe water stress and has made contact with the fungus, however, the extremely rapid rate of growth of *S. bataticola* and its ability to parasitize permit it to be the pioneer colonizer in the host. From the observations that sclerotia or mycelia were not formed to any extent in unsterilized soil away from the food base, it seems that infected plant debris is one if not the principal means of survival of the fungus subterraneously. The cause of the inhibition of *S. bataticola* by other soil microorganisms has not been determined. Although lysis and possibly other forms of parasitism were noted occasionally, it is believed that this plays a relatively minor role in the suppression of linear growth of the charcoal rot fungus. With reference to its saprophytic activity in the soil, *S. bataticola* is placed in Garrett's (6) group of fungi making no extensive spread through the soil.

SUMMARY

The linear growth rates of the charcoal rot organism, *Sclerotium bataticola*, were measured on yeast extract-dextrose agar, in sterile and unsterile soils. In the soil tests, the fungus was cultured in long tubes kept at 25°, 30°, 35° and 40° C. and at soil moisture levels of 5, 10, 15 and 20 per cent.

The optimum temperature for growth of the fungus on agar and in sterile soil was 35° C. The optimum soil moisture level was 5 per cent. However, good growth usually also occurred at 30° C. and at the 10 and 15 per cent moisture levels.

There was very limited growth of the fungus in unsterilized soil, whether a food base was used or not.

It is believed that the hot, dry weather, usually associated with charcoal rot of guayule, favors development of the disease by reducing the vigor of the plant rather than by favoring the growth of the fungus through the soil.

Department of Plant Physiology and Pathology
Texas Agricultural Experiment Station
and
Division of Rubber Plant Investigations, Bureau of Plant Industry, Soils and Agricultural Engineering
U. S. Department of Agriculture
College Station, Texas

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STUDIES OF LEPTOGRAPHIUM ISOLATED FROM WESTERN WHITE PINE [1]

Ernest E. Hubert[2]

Numerous isolations of fungi made in the field during 1949 and subsequent isolations made in the laboratory during 1950 and 1951 from the trunks and roots of Western white pine (*Pinus monticola* Doug.) showing symptoms of pole blight (1, 10), have yielded a fungus which has been referred to *Leptographium* sp., formerly *Scopularia* sp. (9). This fungus has been found in bark lesions of both roots and stems, and, in its later stages, producing a grayish-blue discoloration of the sapwood. The objective of this study was to determine whether *Leptographium* sp. could be considered a primary cause of pole blight and to gain some information regarding phytotoxins produced by this fungus.

DISTRIBUTION.—The first record of collections of *Leptographium* in the Western white pine region was made in 1931 by Rumbold (8) who isolated it from Western white pine in the Coeur d'Alene National Forest of northern Idaho. She identified it as *Leptographium lundbergii* Lagb. and Melin. and stated that the dark conidiophores were found in sheltered places beneath the bark.

Subsequent collections and isolations have been made by various workers studying the pole blight problem. These records were reported by the Division of Forest Pathology (3, 4, 5) and by the University of Idaho (1) from white pine areas in northern Idaho and western Montana, and by the Canadian Laboratory of Forest Biology (1, 7) from the British Columbia areas.

A number of isolations were made during the period 1949-1952 by the author, on Western white pine in areas within and outside the present known areas of pole blight (11). Collections and isolations were made from trees in the East Fork of Potlatch River several miles south of the pole blight area and in the Clearwater area farther south. It is not known whether these collections represent species of *Leptographium* differing from those collected within the pole blight area. Some of the collections appear to be of the same species, resembling closely the characteristics noted for culture No. 77, *Leptographium* sp. on white pine from the Coeur d'Alene National Forest.

In the United States, *Leptographium* has been found on several coniferous and broadleaf hosts, but only on Western white pine and lodgepole pine (*Pinus contorta* Doug. var. *latifolia* Engelm.) in the northern Rocky Mountain region. It has been collected on these 2 species of pine in the British Columbia area (7) and the ascus or perfect stage was collected on white pine slash by Canadian workers. (1).

INOCULATION STUDIES.—The first reported inoculations with *Leptographium* sp. (*Scopularia*) on Western white pine were made in 1949 (3). Gill et al. reported successful laboratory inoculations on the bark of 62 freshly collected, short trunk sections of white pine by means of needle punctures and the application over them of malt agar cultures of the organisms. Marked depressions or sunken areas formed in the smooth young bark. Of the 20 cultures used only those containing *Leptographium* produced lesions. The introduced organisms were reisolated without difficulty.

Inoculation tests made at the University of Idaho in 1950 (1) consisted of stem inoculations on vigorous, potted white pine seedlings (Table 1) using *Leptographium* sp., culture No. 77 grown on malt agar and isolated from a pole-blight-dead white pine in the heavily affected 4th of July Canyon area near Coeur d'Alene, Idaho. Of 23 trees ranging in age from 4 to 20 years and inoculated by means of needle punctures encircling the bark of the stem or root, 20 developed wilting symptoms and later died. Typical lesions or sunken areas developed in the young bark on 8 trees. Reisolations were made and the control trees remained normal. One of the successful trunk inoculations and 4 of 6 root tests were made using the extract from the fungus mycelium containing viable conidiospores (Table 1) and daily pouring this liquid on the soil of the potted tree. Root inoculations proved more successful than trunk inoculations which, perhaps, reflected the more favorable moisture condition surrounding the roots.

Gill and associates in 1951 (5) reported successful infections accompanied by heavy resinosis on many of the pole size white pines in the forest which were artificially inoculated with *Leptographium* sp. After 1 year cankers or lesions were observed on these trees, the sapwood beneath them showed resin infiltration of the tissues and blue stained areas extending radially toward the heartwood. Of 2000 inoculations made on forest trees in 1949, 1045 were made with species of *Leptographium* on 272 trees.

Field inoculations with *Leptographium* sp., culture No. 77, were made on the lower trunks of 20 pole size white pine trees in 1950 by Buchanan, Johnson and Estes (1). When examined in 1951 all the inoculations proved successful, resulting in typical sunken areas and copious resinosis. These inoculations were made by sterile needle punctures in the bark on one side of the trunk and covering an area about 4 sq. in. Examination of the trees in 1952 disclosed that some of the inoculations had produced additional resin flow while others showed little change. The general appearance of the lesions indicated a marked reduction in lesion area spread and an apparent increase in resistance of the bark tissues to the fungus. The greater spread of the lesions, in all cases, was parallel to the stem axis and slightly greater above than below the center of

[1] Accepted for publication August 6, 1953.

[2] Research Pathologist, Forest, Wildlife and Range Experiment Station, University of Idaho. This work carried out under Special Research Project No. 11.

TABLE 1.—*Laboratory inoculations on Western white pine seedlings with Leptographium sp., 1950*

Number of Trees	Age Years	Part Inoculated	Period of Test Days	Results Number of Trees			
				Healthy	Wilted	Lesions	Dead
			Inoculated—Malt agar Culture No. 77[a]				
18	4-5	Root Collar	42-85	0	18	3	18
5[b]	9-20	Trunk	33-55	0	0	5	2[c]
			Controls				
18	4-5	Root Collar	42-65	18	0	0	0
5	9-20	Trunk	33-55	5	0	0	0
			Inoculated—Extract of No. 77 Containing Conidiospores				
6[d]	4-5	Roots	40-73	2	2	0	0
			Controls				
6	4-5	Roots	40-73	6	0	0	0

[a] From roots of pole-blight-dead white pine.
[b] Extract containing spores injected via glass tube into bark of 1 trunk.
[c] Two branches killed above lesions.
[d] Extract containing spores poured daily into soil around roots.

the inoculation area. No crown symptoms developed on the trees in this series of tests.

Additional inoculations were made by the needle stab method in 1951 and 1952 with the above fungus on roots, stems, and branches of young white pines in the greenhouse at the University of Idaho (Table 2). Needle stabs were made to encircle root, stem, or branch; fungus mats containing conidiospores were smeared on the inside of wet strips of cotton which were then wound over the needle stabs and wrapped with masking tape or enclosed in a tight cylinder of cellophane sealed to the bark at the tip and bottom with masking tape. Typical lesions (Fig. 1, left) developed in all cases; branch inoculations also resulted in death whenever a lesion continued to develop and completely girdled the stem. Some of the root and some of the stem inoculations failed to kill the trees. However, the root bearing the lesion, in most cases,

TABLE 2.—*Greenhouse inoculations on Western white pine seedlings, 16-18 years old, with Leptographium spp., 1952*

Number of Roots, Stems or Branches	Part of Tree	Period of Test Days	Lesions and Resinosis Days	Results Number of Trees			
				Healthy	Wilted	Lesions	Dead
			Inoculated—Malt agar Culture No. 77				
10	Roots	90	21-57	0	0	10	5
10[a]	Stems	90	21-57	1	6	9	6
10	Branches	90	36-49	0	10	10	10
			Controls				
10	Roots	90	———	10	0	0	3[b]
10	Stems	90	———	10	0	0	0
10	Branches	90	———	10	0	0	0
			Inoculated—Malt agar Culture No. 77				
5	Stems	70	32-45	0	0	5	0
			Inoculated—Malt agar Culture No. 641[c]				
5	Stems	80	32-50	1	0	4[d]	0
			Inoculated—Malt agar Culture No. 596[e]				
5	Stems	80	32-45	0	0	5	0
			Controls				
5	Stems	80	———	5	0	0	0

[a] Inoculated one tree below needle puncture area.
[b] Three trees died following tests, as a result of root crowding in pots.
[c] *L. serpens* from Baarn, Holland.
[d] Lesions small, restricted to individual needle punctures.
[e] From beetle-killed white pine, E. Fork of Potlatch River.

ceased to develop new rootlets and died. The above inoculations were made by extending the needle punctures completely around the root, stem, or branch in a band from 1 to 1½ in. wide. Heavy resinosis accompanied the lesions almost without exception. Inoculations attempted on young, healthy, uninjured bark yielded no infections. Most of the lesions developed in 30–60 days following inoculation, the young bark tissues below the lesions remaining turgid and green, whereas the tissues above the lesion in girdled trees soon began to shrink and became discolored.

To test the infective power of some *Leptographium* species collected in the pole blight area of northern Idaho and western Montana with similar isolations made from white pines south of Bovill, Idaho and outside the pole blight area, a number of inoculations were made on stems of young trees in the greenhouse. *Leptographium* culture No. 77 was taken from a tree in the pole blight area of the Coeur d'Alene National Forest, while culture No. 596 (Brush Camp No. 54) is from a beetle-killed white pine in the East Fork of Potlatch River drainage south of the pole blight area. A culture of *Leptographium* (*Scopularia*) *serpens* G. Goid. No. 641, obtained from Centraalbureau voor Schimmelcultures, Baarn, Holland, was also used to determine its infective power and for comparison with the 2 local species.

Leptographium sp. (Culture No. 77) produces fairly large, dark brown conidiophores with broom-shaped fruiting heads (Fig. 1,A), bearing large, whitish drops which contain spores held together by a mucous liquid. With age these drops turn yellow to brown. These conidiophores have an average length of 640μ and a width of about 2μ. The spores measure about 3–7 × 2–3 microns. The crown of the fruiting head contains hyaline, branched, and slender members bearing spores at their tips (Fig. 1,B). The stalks have a root-like base and 2 or more stalks may issue from a common base. Conidiophores develop rapidly on and under the bark and on the cut ends of infected seedling stems.

Another culture, No. 596, used in the inoculation experiments closely resembles culture No. 77 but appears to have shorter stalks and narrower fruiting heads.

Results of these tests are in Table 2, lower half. Typical lesions were obtained with culture No. 77. Culture No. 596 inoculations produced lesions similar to those resulting from No. 77 inoculations but were somewhat slower in development. The inoculations with culture No. 641 produced very small brownish areas surrounding the individual needle wounds in the bark. Typical lesions comparable to those obtained with culture No. 77 were not developed.

Symptoms.—The symptoms observed on *Leptographium*-inoculated seedlings of white pine consisted of a slight loss of turgor in the older needles and a wilting of the very young needles; a slow drying of the older needles which became brittle, spirally twisted, and turned gray-green at death. The buds became shrunken and dry, the stem bark dry, wrinkled, and

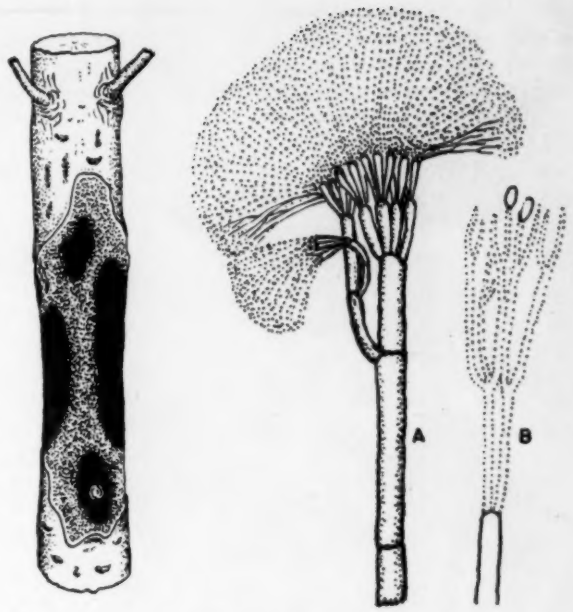

Fig. 1. left: Typical stem lesion produced in the bark of young Western white pine by inoculation with *Leptographium* sp., culture No. 77. This lesion encircles the stem. (× ⅜). right: A) *Leptographium* sp. (Culture No. 77) conidiophore showing branching habit. (× 300). B) Branching of hyaline tip of conidiophore head. (× 900).

grayish-green. Many roots of trees killed by the fungus died and bark slipped easily from them. The first symptoms appeared at about 25–35 days, and death followed 35–40 or more days after inoculation.

The lesions or sunken areas in the young bark of the stem (Fig. 1) consisted of irregular, somewhat fusiform, depressed areas of a red-brown in the central portion fading into the green of the surrounding bark. The margins developed more rapidly in a longitudinal direction above and below the center of infection. The rate of encirclement of the stem or branch was relatively slow. Complete girdling of branch, stem, or root resulted when the needle punctures were made entirely around each part, and in such cases death of the branch, the entire tree, or the root resulted. High moisture conditions, such as wrappings of wet cotton, and enclosure within a cellophane cylinder, favor lesion growth while the dry air of the laboratory and no wrappings appeared to arrest growth. This difference was also noted for greenhouse conditions.

Histology.—Sections cut from the stems of seedlings killed as a result of direct inoculations with *Leptographium* sp. and examined through the microscope showed numerous, very large, dark brown hyphae in the bark tissues and in the xylem. These hyphae, often anastomosed and branched, completely filled many of the ray cells and vertical resin ducts. Numerous hyphae were found in the wood tracheids, most of them parallel to the long axis and some passing through the pit pairs while several were observed penetrating the cell walls in a direct radial line. The

FIG. 2. Effects of fungus extracts on tomato cuttings. LEFT: Control in distilled water. RIGHT: Two cuttings after placing in *Leptographium* sp., extract, 1:2, for 2 days. These show extreme wilting, curling, necrosis, and are dead.

hyphae were constricted at the point where they passed through the cell wall. The young hyphae were small and hyaline, changing color when about 8–10 or more days old. A study of stem cross sections at a point ½ in. above the needle stab area on an artificially produced lesion, showed bark tissue filled with small to large, hyaline to dark brown hyphae. The cambium area was crowded with these hyphae, which spread into and filled the medullary rays of the xylem. The vertical resin ducts were also invaded and hyphae followed the rays into the pith on young branches. Bladder hyphae frequently filled the wood tracheids, and resin deposits were common. The plugging action of the large and numerous hyphae and copious resin in both phloem and xylem tissues may have an important bearing on the cause of death. The symptoms indicate a shutting-off of the moisture supply either through death of the roots or as a result of an impaired xylem function or both.

TOXIN EXPERIMENTS WITH LEPTOGRAPHIUM SP.—A few preliminary experiments were undertaken to determine whether *Leptographium* produced a toxin which might result in symptoms some distance removed from the fungus mycelium. The fungus, culture No. 77, was grown in the laboratory on the surface of a 2.5 per cent concentration of malt agar extract in water. Cork floats were used to support the inoculum and to prevent the thick mycelial mat and spores from sinking below the surface of the liquid. Thick black mats covered with spore masses developed within 40 days. This growth, which developed a sour wort odor (6), was removed, washed in distilled water, drained, and weighed. It was then broken up in a burr mill, ground in a mortar with silica sand, and allowed to soak for 24 hours in known weights of distilled water.

The extracts thus obtained were filtered through cotton, then a #44 Whatman filter paper and finally through a Seitz 0.1μ pore size filter. Streak cultures on malt agar slants were made of these liquids to test them for the presence of bacteria or fungi. Re-filtra-

tion after an incubation period was necessary in the case of *Leptographium* to eliminate all traces of minute conidiospores which passed through the first series of filters. The filtered extracts with a few drops of chloroform added were then stored in a refrigerator until used.

Tomato cuttings were used to indicate presence of phytotoxins and to gain some idea of the effect of various concentrations of the extracts. The extracts producing wilt, curling, and drying of leaf margins and finally death of these cuttings, were then tested against 4-year-old white pine seedlings transplanted into pots and grown in the greenhouse for 2 months before testing. These seedlings were obtained from the Region One Forest Service Nursery at Haugan, Montana. One set of seedlings was moistened daily with equal, measured amounts of the extract for a period of 10 days or until the supply of extract was exhausted. The control set was moistened daily with distilled water.

Used for injecting the extract into pine seedlings, were hypodermic syringes, needle size No. 22, and lengths of glass tubing 0.3–0.7 cm. in diameter, bent at an angle to form a short section which fitted into a hole in the bark made by a cork borer. This hole extended to the sapwood surface.

Results of these experiments, using 32 tomato cuttings and 22 white pine seedlings, show that the various concentrations of *Leptographium* extracts and of extracts with spores, produced wilting and death in 16 fresh tomato cuttings (Fig. 2). The 16 control cuttings placed in distilled water remained normal. The extracts plus spores also caused death of 4 of the 4-year-old Western white pine seedlings with wounded roots. The 1:2 concentration (1 part water to 2 parts of wet mycelium by weight) of the extract curled, wilted, and finally killed the tomato cuttings within 2 days. Severely wilted cuttings removed from the extract and placed in distilled water did not recover. None of the 6 white pine seedlings watered daily with the spore-free extracts or injected into the stems showed any abnormal symptoms after 90 days. Only those 6 seedlings with wounded roots, watered daily with extracts plus spores of *Leptographium*, developed symptoms and died while the controls remained normal. These symptoms consisted of slight loss of turgor in the older needles and wilting of the very young needles, slow drying of the older needles until death of the tree. In the last stages the needles were dry, brittle, and spirally twisted, remaining a gray-green color. The buds were somewhat shrunken and dry. At death the stem showed a wrinkled, dried, gray-green bark. Many roots were dead and the bark slipped on most of them. The first symptoms appeared at about 25–35 days, and death followed 35–40 or more days after inoculation. These symptoms resemble closely those produced by direct inoculations.

These tests of phytotoxic action on pine seedlings are not conclusive since the amount of the extracts absorbed was small (2.6–6.4 c.c.). While the reactions on tomato cuttings were positive, it must be kept in mind that cuttings and seedlings of this indicator plant are very sensitive to toxic solutions. Additional tests are needed to determine the toxic limits.

The symptoms of young trees in the greenhouse inoculated with *Leptographium* sp. and tested for phytotoxins of this fungus do not resemble those described for pole blight. The pole-size trees inoculated in the forest, although developing lesions in the bark, showed no crown symptoms. It appears safe to assume that the species of *Leptographium* so far tested are not responsible for the death of trees showing pole blight symptoms.

SUMMARY

Collections and isolations were made of *Leptographium* spp. developing on Western white pine in association with symptoms of pole blight and on lodgepole pine. Inoculation experiments established the ability of this organism to produce lesions in the bark of its host and to cause death of seedlings under certain conditions. Wounding of the bark proved to be necessary for infection. The production of phytotoxins was demonstrated by means of an indicator plant and preliminary tests with white pine seedlings were conducted.

Similar species apparently differing in virulence were found both within and outside the present known spread limits of pole blight.

The inoculation and phytotoxin tests indicated that *Leptographium* sp. is not a primary cause of death of trees showing pole blight symptoms.

Forest, Wildlife and Range Experiment Station
University of Idaho
Moscow, Idaho

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THE EFFECTS OF CONTROLLED PERIODS OF HIGH AND LOW HUMIDITIES ON THE DEVELOPMENT OF PURPLE LEAFSPOT OF ORCHARDGRASS [1]

J. H. Graham and V. G. Sprague

Purple leafspot of orchardgrass (*Dactylis glomerata* L.), caused by *Stagonospora maculata* (G.) Sprague, is prevalent throughout the Northeastern United States even during periods of low rainfall. This was partially attributed to the capacity of the fungus to grow over a relatively wide temperature range and to the rapidity of penetration of its germ tubes.[2] Studies were continued to determine whether or not newly germinated spores could persist on warm sunny days and then resume growth and penetrate leaves during favorable periods at night.

Inoculation trials were conducted during February 1953 in a greenhouse at State College, Pennsylvania. Plants of a susceptible clone of orchardgrass were sprayed with a spore suspension obtained from diseased, overwintered orchardgrass leaves and then subjected to the following treatments (Fig. 1). The inoculated plants were placed in a moist chamber at 100 per cent relative humidity and at 18°C. for 6 hours. Following this period, 32 plants were kept at 18°C. and an equal number at 24°C. At each of these temperatures, one half of the plants was kept at low relative humidities (30–60 per cent) and the other half at high relative humidities (40–100 per cent). From each of these 4 groups 4 plants were removed after 1, 2, 4, and 6 days. One half of those removed was placed in a moist chamber for 2 days at 100 per cent relative humidity and then moved to a holding room maintained at a relative humidity of 30–60 per cent at 20–24°C. The other half of the plants was moved directly to the holding room without the 2-day treatment at 100 per cent relative humidity. To evaluate the effects of these low and high humidities for different time intervals, 2 plants were maintained at 100 per cent relative humidity for 2 and 8 days before moving to the holding room.

The low relative humidities were those that occurred naturally in the greenhouse and varied from 30–60 per cent. The higher humidities were maintained in another section of the greenhouse by the intermittent use of a water spray and low pressure steam and varied between 40–70 per cent during the day and 90–100 per cent at night. Under these conditions water droplets usually formed on the leaves.

Disease readings were taken at the time of maximum incidence, usually 8–10 days after the last period of high humidity. Since no differences were found in the incidence of disease between plants at 18°C. and those at 24°C. the averages of the 2 temperatures are reported (Table 1). The disease was most severe on the plants that remained in the moist chamber continuously whereas those kept in the low humidity room developed only a few lesions, probably from those penetrations that occurred during the initial 6-hour moist period. In the high humidity room the fungus continued to penetrate the leaves during periods of high humidity at night. One night did not provide sufficient time to increase the disease incidence. After 2 nights the plants were only slightly diseased whereas numerous lesions developed after 4 and 6 nights at high humidities. The incidence of disease that occurred under these conditions was almost as high as that obtained on plants held at 100 per cent relative humidity for 2 days following inoculation.

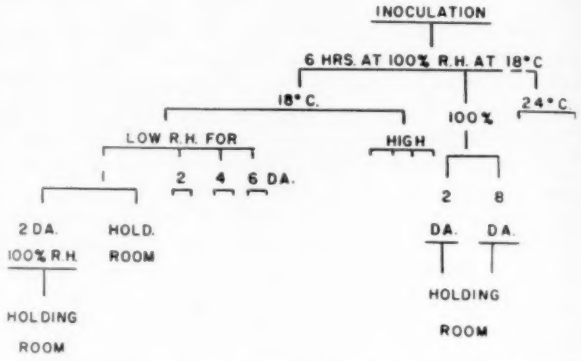

FIG. 1. Treatment of orchardgrass plants following inoculaion with *Stagonospora maculata*.

The plants placed in the low humidity room for 1 and 2 days developed additional lesions after being returned to the moist chamber for 2 days, but after 4 and 6 days at low humidities an extra period of high humidity did not affect disease development. It appeared that the spores and young hyphae had been killed by the drying.

At high humidity, the plants became moderately to

TABLE 1.—*Incidence*[a] *of purple leafspot of orchardgrass in the greenhouse as affected by various periods of low and high relative humidities (R.H.)*

Plant treatment after inoculation and 6 hours at 100% R.H.	Plants removed from low or high R.H. after the following number of days				
	1	2	4	6	8
100% R.H. continuously		8			10
Low R.H. (30-60%)	tr	1	tr	tr	
High R.H. (40-100%)	1	2	6	8	
Low R.H. plus 100% for 2 days	2	3	tr	tr	
High R.H. plus 100% for 2 days	6	6	7	8	

[a] Incidence was rated on a scale of 0 to 10, with 10 indicating most severe disease. All ratings were taken at time of maximum disease incidence.

[1] Accepted for publication August 6, 1953.
Contribution No. 125 of the U. S. Regional Pasture Research Laboratory.

[2] Graham, J. H. 1952. Purple Leafspot of orchardgrass. Phytopathology 42: 653-656.

severely diseased even without the extra 2-day treatment at 100 per cent relative humidity.

The test was repeated 1 month later (March) at a temperature of 22°C., which is optimum for spore germination and infection. Results of this test were similar to those obtained earlier. However, the overall disease incidence was higher in the March test, presumably because of fewer sunny days.

These results may explain the incidence of purple leafspot even during relatively dry periods. If spores are splashed onto healthy leaves by rain and the leaves dry before penetration occurs, growth of the fungus will be halted. Germination and growth will resume if a period of high humidity occurs within approximately 2 days. It is probable that over much of the Northeastern Region this requirement for moisture is fulfilled on most nights during the growing season. Sprague[3] at the Pasture Laboratory has measured the relative humidities among growing plants and has found that a relative humidity of 100 per cent frequently occurs in a forage plant cover for 6 hours or longer at night even during periods of low rainfall. Two successive nights of high humidity are sufficient for moderate disease infection while 4 nights probably would produce epiphytotics equal to those produced after a protracted moist period.

U. S. Regional Pasture Research Laboratory
State College, Pa.

[3] Sprague, V. G. Unpublished data.

PHYTOPATHOLOGICAL NOTES

A Ringspot Virus Disease of Panax.[1] M. Aragaki,[2] H. Murakishi,[2] and J. W. Hendrix.[3] A ringspot disease of panax [*Nothopanax guilfoylei* (Cong. and Merch.) Merr.; synonym, *Polyscias guilfoylei* (Bull) L. H. Bailey], commonly used as a screen and hedge plant in tropical regions, has become prevalent in Hawaii. The disease was first noticed in Honolulu in 1948.[4] A review of available literature revealed there were no similar disorders of panax otherwise recorded. The symptoms of the disease (Fig. 1) are concentric rings on the leaves, premature leaf casting, and stunting of the plants. The ringspots start as tiny chlorotic areas about 1 mm. in diameter. As the disease progresses concentric rings are formed, the centers turn white and the outer edges of the rings become purplish-brown. The average diameter of the spots at this stage is about 6 mm., although some unusually large spots may measure up to 20 mm. across. The leaflet casting is not generalized, although occasionally nearly complete defoliation has been observed in individual plants.

Fifty cuttings were obtained from a diseased panax hedge and rooted individually in cans. Within 2 months all the plants showed ringspot symptoms on the new leaf growth, whereas none of the cuttings from apparently healthy plants propagated at the same time produced disease symptoms. One hundred per cent transmission was obtained by side grafting diseased scions on healthy stock (10 out of 10), healthy scions on diseased stock (8 out of 8), and by inarching (5 out of 5). A comparable number of grafted check plants showed no symptoms.

Fig. 1. Naturally-infected panax leaflet showing ringspot patterns.

Other methods of transmission met with failure. Attempts to transmit the virus by juice inoculation to healthy panax gave negative results. Aphid transmission tests with *Myzus persicae* Sulz. and *Aphis gossypii* Glov. were also unsuccessful. *A. gossypii* severely curled and distorted the leaves they fed on, but after the aphids were killed, the subsequent new

[1] Published with the approval of the Director of the Hawaii Agricultural Experiment Station as Technical Paper No. 293.

[2] Assistant-in-Plant Pathology, and Associate Plant Pathologist, respectively, Hawaii Agricultural Experiment Station.

[3] Formerly Associate Plant Pathologist, Hawaii Agricultural Experiment Station. Present address, Washington State College, Pullman, Washington.

[4] Hendrix, J. W. Unpublished notes. Hawaii Agr. Exp. Sta. 1948.

growth was normal.

Attempts were made to transmit the virus to tomato, nasturtium, and *Nicotiana glutinosa* by inserting diseased panax tissue into slits made in the stems, but no symptoms appeared.

N. guilfoylei seldom produces seed in Hawaii, so that the only method of propagation is by vegetative cuttings. Careful selection of planting material has aided in establishing a healthy panax hedge on the University campus.—University of Hawaii, Agricultural Experiment Station, Honolulu, Hawaii.

Compatibility Types of the Oak Wilt Fungus in 23 Appalachian Trees. JOHN S. BOYCE, JR. AND KENNETH H. GARREN.[1] Compatibility types of the oak wilt fungus, *Endoconidiophora fagacearum* Bretz, can be determined readily.[2] It was thought that by isolating the fungus from representative parts of diseased trees and determining the compatibility type of each isolate, indications might be obtained as to where infections had occurred in the trees. Accordingly, studies were made of 23 trees affected with oak wilt, belonging to the red oak group, felled in western North Carolina and eastern Tennessee during the summer of 1952.

Branch and trunk samples for culturing were obtained immediately after the trees were felled, the former from each of the main branches, the latter from 3 points around the circumference of discs sawed out at 6- to 10-foot intervals from the stump to the crown. A map showing the location from which each sample was taken was prepared for each tree.

Nutramigen agar[3] was employed for all culturing. Isolates of *Endoconidiophora fagacearum* were stored for 3–5 months in a refrigerator until time permitted making fresh transfers of them for compatibility testing. Each unknown isolate was spermatized[2] by conidia from known cultures of type A and type B compatibilities.

Table 1 summarizes the results. The oak wilt fungus was not isolated from all places sampled in these trees. Wilt symptoms, including defoliation and leaf discoloration, were pronounced for all trees when felled except tree 13, which was in an early stage of wilt. Of 727 isolation plates made from 23 trees with oak wilt, 253 yielded *Endoconidiophora fagacearum*, 46 were contaminated beyond identification of the fungus, and 428 were sterile. Each plate contained 5 serial wood chips representing 1 place in a tree. Failure to obtain the fungus in so many of the cultures emphasizes the advisability of culturing wood from several places in a tree suspected of having oak wilt.

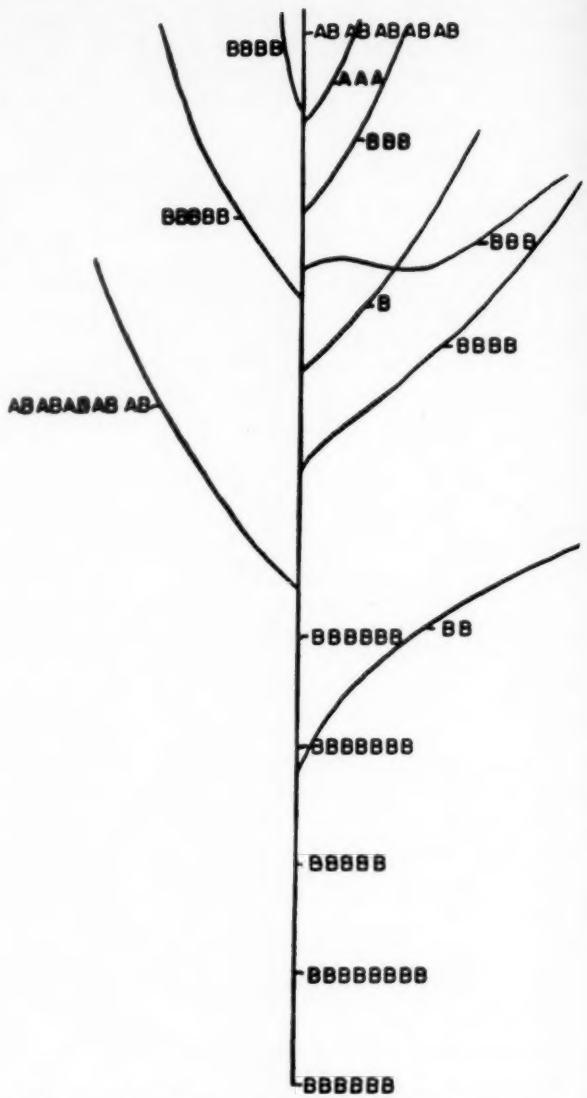

FIG. 1.—Distribution of *Endoconidiophora fagacearum* compatibility types in tree no. 3. Each letter and the combination AB represents 1 isolate.

Twenty-two of the 23 trees sampled contained only 1 compatibility type of *Endoconidiophora fagacearum* within a given tree. Tree nos. 7, 10, 11, and 21 were beyond root-graft distance from possibly infected trees, whereas the rest were within 50 ft. of wilting trees, dead standing trees, or stumps. Six trees yielded type A isolates and 16 trees type B isolates, showing that both compatibility types of the fungus are active in infections in this area. Only 1 com-

[1] Respectively, Associate Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, Asheville, N. C., in cooperation with the Southeastern Forest Experiment Station, U. S. Forest Service; and Associate Professor, Alabama Polytechnic Institute, Auburn, Ala.

[2] Hepting, G. H., E. R. Toole, and J. S. Boyce, Jr. 1952. Sexuality in the oak wilt fungus. Phytopathology 42: 438-442.

[3] Nutramigen agar: Nutramigen (Mead Johnson and Co.) 20 grams; agar 15 grams; water 1,000 cc.

TABLE 1.—*Occurrence of oak wilt fungus compatibility types in 23 Appalachian trees*

Tree number[a]	Tree D.B.H.[b]	Cultures made from crown				Cultures made from trunk			
		Places cultured	Compatibility types A	B	AB	Places cultured	Compatibility types A	B	AB
	Inches	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
21	24.3	34	14	0	0	36	1	0	0
24	24.0	0	0	0	0	33	0	9	0
1	21.5	10	0	7	0	15	0	7	0
23	20.5	19	9	0	0	18	2	0	0
18	19.3	19	11	0	0	18	6	0	0
20	19.0	12	0	1	0	24	0	7	0
2	17.3	11	0	8	0	15	0	1	0
3	15.8	11	1	7	2	15	0	9	0
11	15.0	13	0	9	0	18	0	10	0
15	15.0	14	0	4	0	18	0	3	0
10	14.0	16	0	10	0	18	0	2	0
19	13.6	17	0	15	0	18	0	5	0
7	13.5	10	0	5	0	15	0	6	0
5	13.2	10	0	1	0	15	0	2	0
4	11.3	11	0	2	0	15	0	1	0
16	10.6	10	0	3	0	18	0	2	0
12	10.3	14	5	0	0	18	7	0	0
17	9.8	9	0	3	0	18	0	4	0
22	9.4	16	3	0	0	18	10	0	0
13	9.2	15	0	1	0	18	0	3	0
14	8.6	14	11	0	0	18	7	0	0
9	5.0	15	0	5	0	9	0	5	0
8	4.0	10	0	2	0	9	0	3	0

[a] Trees located within 50 feet of each other: group 1—1, 19; group 2—3, 4, 5; group 3—2, 13, 20; group 4—15, 16, 17.
[b] Tree diameter 4½ ft. above ground.

patibility type of the fungus was isolated from a given group of trees that were within root-graft distance of one another.

Tree no. 3 contained both compatibility types, with a significant distribution (Fig. 1). Type B occurred throughout the stem and in most of the branches sampled. Type A was isolated alone from a single branch in the upper crown and, in mixture with type B, from 2 branches. Tree no. 3 was located 15 ft. from tree no. 4, and 55 ft. from tree no. 5, both of which gave type B isolates. These 3 trees were within root graft distance of dead oaks that probably died in 1951. Root connections between these trees and tree no. 3 may have served to infect this tree with the type B fungus.

It is almost certain that the A type could have been introduced into the tree only by 1 or more inoculations in the upper crown, because it was not isolated from the main stem. Tree no. 14, which was also infected with type A, was about 600 ft. distant in the same woodlot.

The observation that crown infection of tree no. 3 probably took place is noteworthy because it has been suggested that certain insects may inoculate parts of tree crowns.[4]

[4] Craighead, F. C. and C. L. Morris. 1952. A progress report: Possible importance of insects in transmission of oak wilt. Pennsylvania Forests and Waters 4: 126-129.

Rhizoctonia solani Infections of Potato Tubers in India. M. J. THIRUMALACHAR.[1] *Rhizoctonia solani* Kuhn. inciting black scurf disease of potato occurs extensively in the plains and hills of India. The sclerotia borne on the tuber surface as brown encrustations, eventually bring about cankering of stem, root rot, and girdling of shoots, resulting in development of aerial tubers and other characteristic symptoms. The basidial stage, *Pellicularia filamentosa* (Pat.) Rogers is formed on basal parts of haulms during the month of January in the plains of India when cool weather conditions favor its development. The sclerotial stage on tubers causes no direct damage, since the sclerotia are borne superficially without penetrating the tuber tissues. However, 2 instances of tuber infections by *R. solani* have previously been reported. Ramsey[2] reported a form of potato disease which he termed 'dry core' incited by *R. solani*. The hyphae penetrated lenticels and formed crater-like depressions on the tuber surface. Thatcher[3] described a stem-end rot of potato as being due to *R. solani* infection. In the present study 2 types of tuber infections incited by *R. solani* have been investigated, one of them being identical with the 'dry core' disease described by Ramsey and the other of a type not previously reported.

A dry core type infection has been noticed on a large scale in the variety of O. N. 45 at the Central Potato Research Institute, Patna. The tubers are

[1] The writer wishes to acknowledge his indebtedness to Dr. S. Ramanujam, Director, Central Potato Research Institute, for valuable suggestions and encouragement.

[2] Ramsey, G. E. 1917. A form of potato disease produced by Rhizoctonia. Jour. Agr. Res. [U.S.] 9: 421-426.

[3] Thatcher, F. S. 1942. A stem end rot of potato tubers caused by Rhizoctonia solani. Phytopathology 32: 727-730.

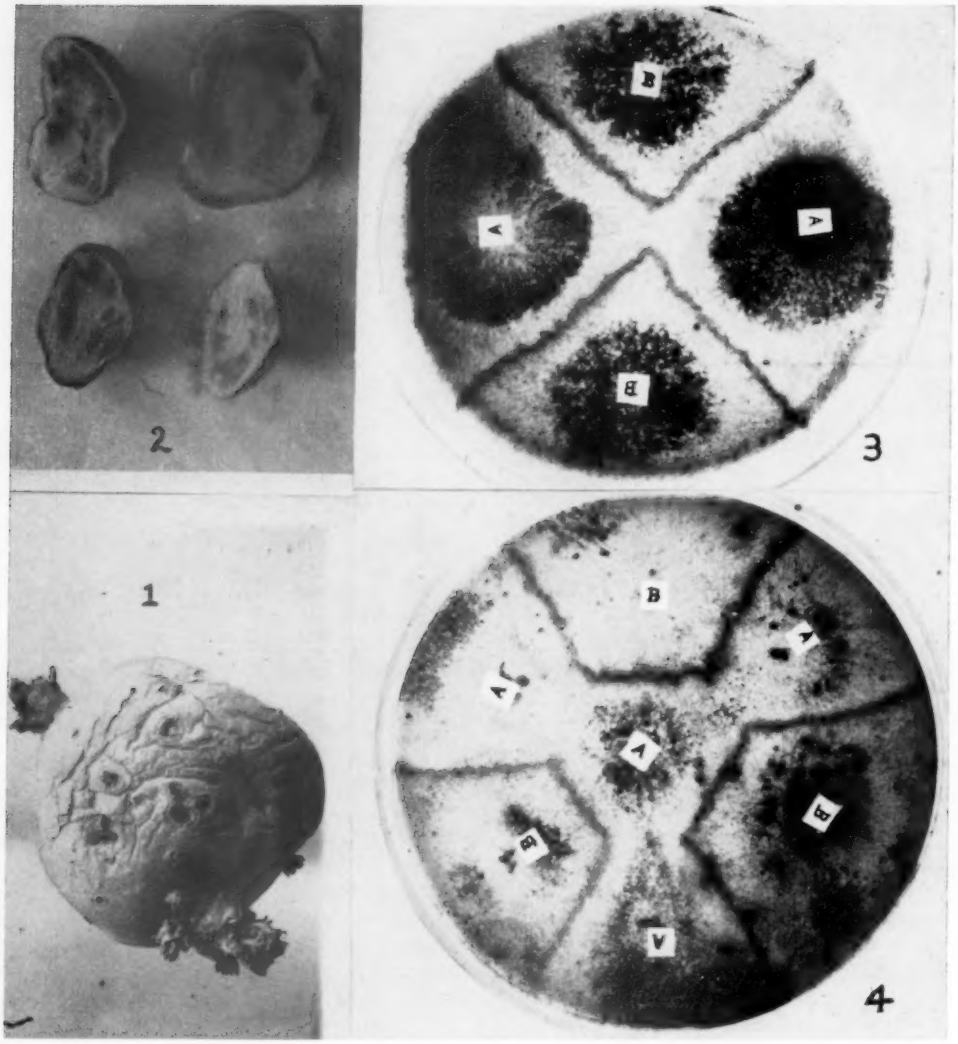

FIG. 1. Crater-like depressions on tuber × nat. size.
FIG. 2. Brown dry rot type of infection in Darjeeling Red Round tubers × ½ nat. size.
FIG. 3 and 4. Plate cultures showing aversion reaction among the isolates.

large and usually show heavy infestation with sclerotia of *R. solani*. In about 5 per cent of tubers, crater-like depressions, which disfigure the tubers and reduce their market quality, have been noticed. Microscopic studies indicated that the hyphae first penetrate the proliferated lenticels. The infected spot becomes slightly swollen and pustulate. In due course, the affected portions dry up and are converted into a powdery mass, which ultimately leaves a crater-like depression, 5–10 mm. wide and bordered by a ridge (Fig. 1).

The second type of infection by *R. solani* has been noticed in the variety Darjeeling Red Round grown at the Potato Certification sub-station, Kufri, which is located at an altitude of 8500 ft. above sea level in the Himalayas. Superficially the infected tuber is easily mistaken for late blight infection, since it shows a hard dry rot with browning inside (Fig. 2). During the storage period, the entire tuber becomes converted into a spongy mass and very light in weight. A rough estimate of percentage of infection in tuber lots received from Kufri is 5–10 per cent.

Microscopic examination and cultural studies indicated that the fungus is a strain of *R. solani*. The hyphae and sclerotia produced in artificial culture are golden brown and differ from those isolated on tubers in the plains. Several isolates of the fungus from within the tuber tissue were made and compared for differences in cultural characteristics and pathogenicity. Some of the isolates when plated on potato dextrose agar media in different combinations showed the aversion phenomenon by the formation of a thick band of sclerotial mass (Fig. 3 and 4). The significance of the aversion phenomenon has been described

by Vandendries and Brodie[4] and Vorhees[5] who showed it to be due to differences in genetic constitution of the mycelia. In the *R. solani* under study, the different physiological strains were distinguished on the basis of aversion.

Inoculation experiments were carried out using artificial cultures of the fungus. Twenty-five Darjeeling Red Round tubers were inoculated with the fungus by needle puncture, and 25 tubers were kept as controls. The inoculated tubers were incubated for 10 days at 15° C. after which they were transferred outside and incubated at room temperature (20–24° C.). After 15 days the tubers were cut at the point of inoculation to observe the spread of fungus from the point of inoculation. In 20 of 25 tubers, the fungus had spread from the point of inoculation, inciting browning and dry rot. In all the controls which were wounded, but not inoculated, the wounded portion healed off and the tubers remained healthy.—Central Potato Research Institute, Patna, Bihar, India.

[4] Vandendries, R. and Brodie, H. J. 1933. Nouvelles investigations dans le domaine de la sexualite des Basidiomycetes et etude experimentale dans barrages sexuele. Cellule 42: 165-209.

[5] Vorhees, R. K. 1942. Life history and taxonomy of the fungus Physalospora rhodina. Fla. Agr. Exp. Sta. Bul. 371, p. 91.

Resistance in Kenaf to Colletotrichum hibisci.[1] J. B. Pate.[2] It has been demonstrated[3] that *Colletotrichum hibisci* Poll.[4] causes a serious disease of kenaf, *Hibiscus cannabinus* L., which menaces commercial plantings of the crop in South Florida. This paper reports the development of lines of kenaf that seem to be highly resistant to the disease caused by this fungus.

Pure line selection in kenaf was initiated in 1951 for development of varieties adapted to South Florida. In the initial stages of the breeding program particular attention was given to selection within the El Salvadorian variety which has been grown on a limited commercial scale in Florida and Cuba. This variety is highly variable, and though susceptible to a number of diseases,[5] has given good yields when grown in the absence of these diseases.[6]

[1] Published with the approval of the Chief, Bureau of Plant Industry, Soils and Agricultural Engineering.

[2] Research agronomist, Division of Cotton and Other Fiber Crops and Diseases, B.P.I.S.A.E., Agricultural Research Administration, U.S.D.A.

[3] Presley, J. T. 1952. Colletotrichum tip blight of kenaf: a brief history of the disease. U. S. Dept. Agr. Pl. Dis. Reptr. 36: 333-334.

[4] Identified by J. A. Stevenson, Head, Division of Mycology and Disease Survey, B.P.I.S.A.E., U.S.D.A.

[5] Stoner, W. N., F. V. Stevenson, W. G. Genung, W. H. Thames, Jr., C. C. Seale, E. O. Gangstad and J. B. Pate. 1952. Preliminary reports of some of the disease and pest problems on kenaf, Hibiscus cannabinus L., in South Florida. U. S. Dept. Agr. Pl. Dis. Reptr. 36: 121-126.

[6] Seale, C. C., J. F. Joyner, and E. O. Gangstad. 1952. The experimental culture of kenaf, Hibiscus cannabinus L., for fiber and seed in South Florida. Turrialba. 2: 99-105.

Fig. 1. Plants of the El Salvadorian variety of kenaf 1 week after inoculation with *Colletotrichum hibisci.*

Since the disease did not develop in nursery plantings in 1951, there was no opportunity to select for resistance. However, apparently resistant open-pollinated plants were selected in severely diseased commercial plantings of the El Salvadorian variety. This variety and the open-pollinated selections were planted in a spring nursery in 1952. An epiphytotic of adequate severity to permit rigorous selection for resistance did not occur, and artificial inoculations with a spore suspension of *C. hibisci* were made. Plants were inoculated by using a hypodermic needle to pierce the stem 1–2 in. below the terminal bud and applying a drop of inoculum to the wounded surface.

Two distinct classes of plants, resistant and sus-

Table 1.—*Reaction of the El Salvadorian variety of kenaf and selfed selections following artificial inoculation with Colletotrichum hibisci*

Parent stock	No. of plants Resistant	Susceptible
El Salvadorian variety	198	640
Resistant selections	175	44
First generation selfed progenies	**No. of progenies from plants: Resistant**	**Susceptible**
Non-segregating progenies:		
HR[a]	30	0
R	6	2
S	0	1
CS	0	17
Segregating progenies:		
HR-R	34	0
HR-S	7	0
HR-CS	9	2
R-S	0	1
R-CS	0	3
S-CS	0	13
R-S-CS	0	9

[a] HR—highly resistant; R—resistant; S—susceptible; CS—completely susceptible.

FIG. 2. Selfed progenies of kenaf completely susceptible and highly resistant to *C. hibisci*.

ceptible, were noted after the inoculations. In susceptible plants, typical lesions appeared 3–4 days after inoculation and frequently resulted in death of the terminal bud (Fig. 1). In resistant plants, a callused wound similar to pierced uninoculated check plants developed. Resistant plants were reinoculated to detect escapes from the first inoculation.

In the El Salvadorian variety, 24 per cent of the plants were resistant, whereas 80 per cent of the plants in the open-pollinated selections were resistant (Table 1). These results indicate the effectiveness of selection for resistance under conditions of a severe epiphytotic of the disease.

Resistant and susceptible selfed selections made in the 1952 spring nursery were included in the 1952 fall nursery. Plants were inoculated twice with *C. hibisci* using the hypodermic needle technique. Plants were again classified resistant or susceptible on the basis of lesion formation following inoculations. Subsequent observations revealed that the terminal bud continued to grow in some of the susceptible plants. These plants represented an intermediate response to the disease. Therefore, all plants were reclassified into 4 classes: highly resistant, resistant, susceptible and completely susceptible, designated HR, R, S and CS, respectively. HR plants did not show any symptoms of the disease, either immediately after inoculation or later in the season. The intermediate plants were separated into R and S classes on the basis of growth after the onset of symptoms. In R plants the terminal bud continued to grow, though some of the seed capsules were usually affected. In S plants there was less growth after the onset of symptoms and severe capsule infection. In CS plants the terminal bud died after inoculation.

Approximately 1/3 of the progenies from resistant plants were homozygous HR while a few were apparently homozygous R (Table 1). Other progenies from resistant plants were segregating either R, S, or CS plants, but in each progeny HR plants were recovered. Progenies from susceptible plants were more variable, but a majority of them were either homozygous CS or segregating S-CS and R-S-CS. HR and CS progenies are shown in Fig. 2.

The results of these studies indicate that lines of kenaf highly resistant to *C. hibisci* can be developed by pure line selection within the El Salvadorian variety. Completely susceptible lines have also been isolated. There are indications of intermediate classes of resistance and the isolation of true breeding intermediate lines. Studies are underway more specifically to define intermediate classes of resistance. Inheritance of the factors of resistance is being studied in crosses of highly resistant and completely susceptible lines.—Everglades Experiment Station, Belle Glade, Florida.